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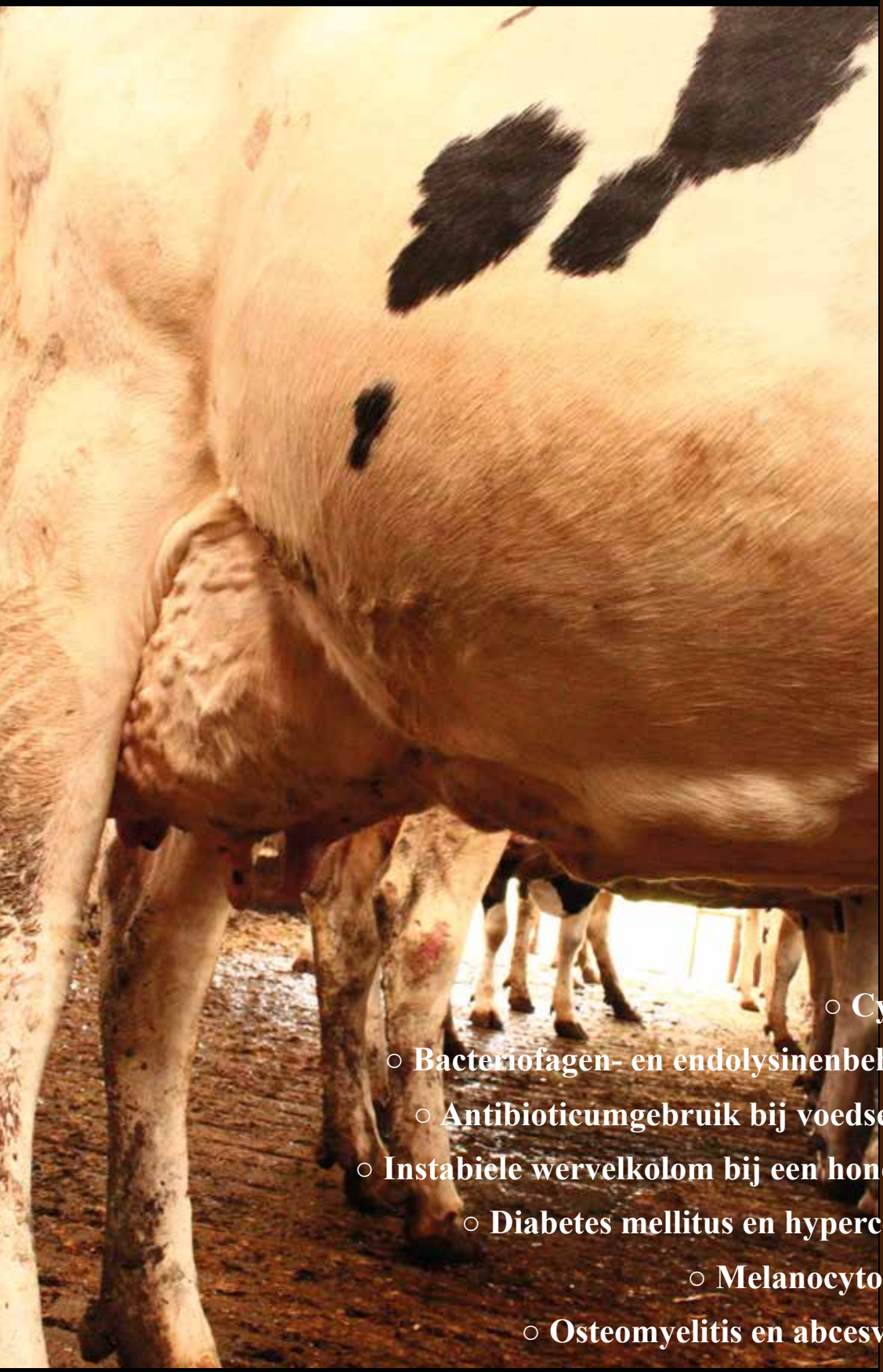

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VLAAMS DIERGENEESKUNDIG TIJDSCHRIFT

2018, vol. 87, nr. 4

INHOUD

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Mastitis is de meest voorkomende ziekte die melkvee treft en daardoor de belangrijkste oorzaak van productieverliezen in de wereldwijde zuivelindustrie. Het overmatig gebruik van curatieve en preventieve antibiotica in deze sector brengt een reëel risico met zich mee voor het ontstaan en de toename van ***antimicrobiële resistentie*** bij mastitispathogenen. Bovendien zijn de traditionele antimicrobiële middelen vaak ineffectief en leiden ze tot residuen in de melk die consumenten van zuivelproducten kunnen schaden. Bacteriofagen (bacterie-infecterende virussen) en bacteriofaag-afgeleide enzymen, de zogeheten endolysinen, worden daardoor momenteel (her)onderzocht in de behandeling van boviene mastitis (cf. pg. 181).

Tekst: Niels Vander Elst

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Potential therapeutic application of bacteriophages and phage-derived endolysins as alternative treatment of bovine mastitis

Potentiële therapeutische toepassing van bacteriofagen en faag-afgeleide endolysinen als alternatieve behandeling van boviene mastitis

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ABSTRACT

The increase in bacterial drug resistance causes major difficulties in the clinical treatment of a growing number of bacterial infections worldwide. Consequently, there is an urgent need to develop novel anti-bacterial agents to control these resistant pathogens and to complement the currently used antibiotics. Mastitis is the most prevalent disease impacting dairy cattle, and therefore one of the costliest diseases in the global dairy industry. The excessive use of curative as well as preventive antibiotics in this sector entails a real risk for the emergence of antimicrobial resistance. Moreover, these traditional antimicrobial agents are often ineffective and lead to residues in the milk, which can affect dairy product consumers. As an alternative therapeutic approach, bacteriophages and phage-encoded endolysins have been proposed and are currently (re)investigated as potential antibacterial agents against mastitis.

SAMENVATTING

De toename van antimicrobiële resistentie veroorzaakt wereldwijd grote problemen bij de klinische behandeling van een groeiend aantal bacteriële infecties. Daardoor is er een dringende behoefte aan nieuwe antibacteriële middelen als aanvulling op de huidige antibiotica om deze resistente pathogenen onder controle te houden. Mastitis is de meest voorkomende ziekte bij melkvee en veroorzaakt de grootste economische verliezen in de mondiale zuivelindustrie. Het overmatig gebruik van curatieve en preventieve antibiotica in deze sector brengt een reëel risico met zich mee voor het ontstaan van antimicrobiële resistentie. Bovendien zijn deze traditionele antimicrobiële middelen vaak ineffectief en leiden ze tot residuen in de melk die negatieve gevolgen hebben voor de consument van zuivelproducten. Als alternatieve therapeutische benadering worden momenteel bacteriofagen en faag-gecodeerde endolysinen (her)onderzocht als potentiële antibacteriële middelen.

INTRODUCTION

Mastitis

Mastitis is an inflammatory process of the udder in response to an intramammary infection, which can have either a clinical or subclinical outcome. In clinical mastitis, the cow is generally ill (e.g. fever, depression) and the affected udder quarter shows redness and swelling. Milk derived from the inflamed quarter often looks abnormal. In contrast, no visual abnormalities are present in the milk derived from an udder quarter with subclinical mastitis. Therefore, subclinical mastitis can easily be diagnosed through measuring an increase of inflammatory cells (somatic cell count, SCC) in the milk (Sadek et al., 2017; Pyörälä, 2003). Other diagnostic methods are the California mastitis test (CMT) and in-line electrical conductivity tests (IECT) (Ruegg, 2002). The CMT involves a colorimetric reaction with the DNA of the inflammatory cells in milk, whereas the IECT measures the electric resistance of milk.

The main cause of mastitis is the penetration of bacteria, yeasts and/or fungi through the teat orifice.

Today, more than hundred organisms are known to cause bovine mastitis (De Vliegher, 2017). Two main approaches are used to stratify these pathogens. A first classification is cow-adapted versus environmental pathogens. Cow-adapted germs survive and propagate mainly on the animal and are transmitted through direct or indirect contact. The most important cow-related bacteria are: non-aureus *Staphylococci spp.*, *Staphylococcus aureus* (*S. aureus*) and esculin-positive cocci. Their prevalence in Flanders is shown in Table 1 (Piepers et al., 2007). Other important cow-adapted bacteria are *Streptococcus dysgalactiae* (*S. dysgalactiae*), *S. agalactiae* and *Trueperella pyogene*s, although their prevalence is significantly lower (Gill et al., 2006; Piepers et al., 2007) (Table 1). In contrast, environmental germs survive mainly in the stable. These pathogens are harder to eradicate and are therefore considered more important than the cow-adapted germs. *Escherichia coli* (*E. coli*), *Klebsiella* and *S. uberis* infections are most common and arise from manure, wood shavings and straw, respectively (De Vliegher, 2017; Gonggrijp et al., 2017). A second classification differentiates 'major' from 'minor' pathogens. Major pathogens cause a high increase in milk SCC and severe clinical mastitis, whereas these characteristics are rather mild in so-called minor pathogens. In contrast to the first classification, this approach does not include the spread of the different pathogens on a dairy farm. Although the latter aspect is important in breaking the transmission cycle, the first approach is internationally more preferred (De Vliegher, 2017).

The data summarized in Table 1 (Piepers et al., 2007) probably have become outdated, as the percentage of cow-adapted infections can be expected to have decreased during the past decade due to more effective control campaigns in Flanders. Indeed, compared to the distribution of the main udder pathogens isolated from clinical milk samples in 2014 (data from the Milk Control Centre of Flanders (MCC Vlaanderen)), the following two observations are important: 1. a decrease in the cow-adapted non-aureus *Staphylococci spp.* and *S. aureus* from 19.1% to 10.6% and 56.6% to 7.8%, respectively; 2. an increase to 18.1% and 18.7% for *S. uberis* and *E. coli*, respectively. These results indicate that in Flanders, environmental-bound pathogens have become more important than cow-adapted pathogens.

Table 1. Prevalence of mastitis and the isolated bacteria in Flemish dairy farms from 2000 till 2002 (Piepers et al., 2007).

	2000	2001	2002	Overall
Number of cows sampled	16,432	16,270	11,965	44,667
% culture positive cows	39.0	41.7	43.0	41.1
Number of quarters sampled	65,728	65,080	47,860	178,668
No. Of culture-positive samples	10,602	11,236	8,637	30,475
% quarters culture-positive for:				
Staphylococcus aureus	19.1	19.1	16.6	18.4
Esculine-positieve cocci	15.5	14.8	18.0	16.0
Streptococcus dysgalactiae	2.8	2.4	1.8	2.4
Streptococcus agalactiae	0.3	0.3	0.3	0.3
non-aureus staphylococci	56.6	57.5	57.4	57.2
Corynebacterium bovis	0.9	1.0	0.6	0.8
Coliforms	1.0	0.6	0.9	0.8
Contaminated samples	2.9	3.4	4.0	3.4
Other	1.0	1.0	0.3	0.8

Bovine mastitis is typically treated with antibiotic preparations, which are administered intramammarily. A division between preparations for dry and lactating cows is consistent in the currently used drugs. In this article however, the possible application of phages and phage-derived endolysins in the future treatment of bovine mastitis is focussed. Current treatments have been described by Royster E. and Wagner S. (2015).

Bacteriophages and phage-derived endolysins

Bacteriophages, or shortly phages, are viruses that infect bacteria. Each phage can infect only one bacterial species (or even strain) because it recognizes an adhesion molecule on the cell wall of the bacterium. When the phage is attached, it infects its host by injecting its genetic material through the production of endolysins. These endolysins are enzymes, which are capable of digesting the bacterial cell wall. Subsequently, there are two possibilities: 1. the phage genome can pass a lysogenic cycle, in which the genome is incorporated into the bacterial DNA (prophage) and will replicate together with the bacterium, 2. the phage genome can start a lytic cycle, in which it uses the bacterial replication mechanisms to multiply its DNA (Figure 1). The genome then assembles with the viral proteins to create a new virion. Due to the massive production of virus particles, the bacterium eventually undergoes lysis. It is this feature which gives phages their ability to kill bacteria and therefore, they are proposed as an alternative to antibiotics. Phage-derived endolysins have also been suggested as a novel antimicrobial agent, because of their ability to lyse bacterial cell walls (Weber-Dąbrowska, 2016).

The One Health Initiative

The One Health Initiative is a movement to forge co-equal, all-inclusive partnerships between physicians, veterinarians, and other scientific-health related experts, recognizing that the health of people is connected to the health of animals and the environment (http://www.onehealthinitiative.com/). The overuse of antibiotics in dairy farming leads to resistant bacteria and antimicrobial residues in milk (Oliver et al.,

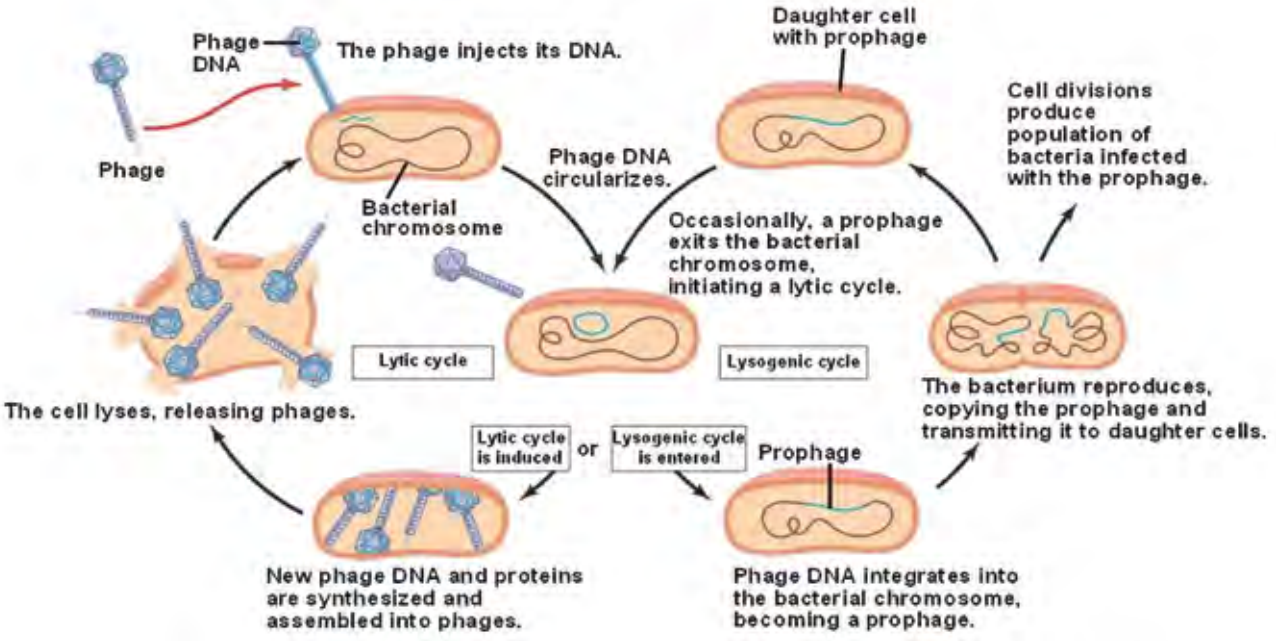

Figure 1. The lysogenic and lytic cycle of bacteriophages. The lysogenic and lytic cycle can pass into one another (https://kullabs.com/classes/subjects/units/lessons/notes/note-detail/8287).

2012). If zoonotic pathogens become resistant, they can no longer be treated with conventional antibiotics when causing disease in humans. Residues of antimicrobial agents in milk and/or meat cause an increase of resistance genes in the commensal intestinal flora. If this resistance is plasmid-mediated, the intestinal flora is considered a source of resistance to possible pathogens (Carattoli et al., 2013).

Methicillin resistant *S. aureus* (MRSA) is known to cause mastitis, but also skin, soft tissue, bone, joint and implant infections, pneumonia and septicemia in both humans and animals. Approximately 20-30% of the human population carries MRSA asymptomatically, resulting from nosocomial or community-acquired transmissions. An important transmission route of community-acquired MRSA is through contact with intensively antibiotic-treated livestock. Close contact with veal calves and pigs is a major risk factor for the transmission of livestock-associated MRSA to humans, because its prevalence is highest in these production animal sectors. In veterinary medicine, MRSA causes its most significant economic losses in the context of bovine mastitis (Holmes et al., 2011; Bosch et al., 2013).

A MINI-REVIEW OF THE BACTERIOPHAGE AND BOVINE MASTITIS LITERATURE

Since bacteriophages and phage-derived endolysins were first proposed as a new potential antimicrobial drug in the treatment of bovine mastitis, promising literature has been published. For this mini-review, the results from approximately ten international peer-reviewed papers are included. Although in vitro and in vivo tests with bacteriophages or phage-derived endolysins have only seldom been reported to treat bovine mastitis, this strategy has been intensively discussed over the past five years, which is indicative of the current and growing interest in bacteriophages and endolysins as alternatives for the traditional antimicrobial agents.

In vitro and in vivo evaluation of bacteriophages in the context of bovine mastitis

Since 2010, several lytic phages derived from mastitis-affected cows have been proposed as potential therapeutic drugs (Han et al., 2013; Kwiatek et al., 2012). The phages are able to effectively infect and kill bovine *S. aureus* including methicillin-resistant strains in vitro. Both bacteriophages are morphologically classified as Myoviridae and exhibit 1. rapid adsorption, i.e. the time needed for free phages to attach to the bacterium, 2. a short latent period, i.e. the time needed for lytic infection, and 3. a relatively small burst size, i.e. the average number of phages needed to cause bacterial lysis. Due to these three characteristics, the bacteriophages have been found eligible for therapeutic use. Another *S. aureus* bacteriophage (*SPW*-phage), also belonging to the Myoviridae family, has recently been isolated from lactating dairy cattle (Li et al., 2014). The three previously mentioned characteristics were likewise evaluated, whereby this *SPW*-phage may indeed have a potential use in future *S. aureus* mastitis therapy.

Recently, a cocktail consisting of four different bacteriophages has been proposed against *E. coli* through several in vitro tests (Porter et al., 2016). A 3.3 to 5.6 log reduction of growth in raw milk was observed when *E. coli* was co-incubated with this phage cocktail for twelve hours. Moreover, bacterial growth decreased with 1.6×10^3 CFU/mL (colony forming

units/mL) when tested against a mastitis-derived *E. coli* strain.

A phage K solution was administered intramammarily (1.25×10^{11} PFU/mL (plaque forming units/mL)) to 24 lactating Holstein cows with a persistent *S. aureus* infection (Gill et al., 2006). While none of the negative control saline-treated quarters were cured, *S. aureus* could not be isolated in only three of the eighteen phage-treated quarters samples, which were consecutively collected at 2 to 7 days, 9 to 14 days, 16 to 21 days, and 23 to 28 days after the end of local phage treatment. The success rate of this phage K therapy is therefore regarded as limited.

A selected phage cocktail for the treatment of *S. aureus*-associated (Newbould 305) bovine mastitis has recently been tested in vitro and in a mouse model (Breyne et al., 2017). First, different cultures were verified in the presence or absence of IgG and the phage cocktail. *Staphylococcus aureus* could not be isolated from any of these cultures to which the phage cocktail was added, whether or not in the presence of IgG. These promising in vitro results were partly confirmed in an in vivo pilot study using a mouse model for bovine mastitis. Mammary glands of lactating mice were inoculated with the same bovine mastitis isolate (N305) of *S. aureus*. Subsequently, a first group of mice was inoculated intramammarily with cefalonium, a first-generation cephalosporin (used as positive control). A second group of mice received no treatment, but only saline (negative control). A third group was injected with the phage cocktail. Breyne et al. (2017) reported that *S. aureus* could not be isolated from the mice which were injected with cefalonium. In contrast, *S. aureus* was still present in the phage-treated group although the number of colony forming units (CFU) was significantly lower when compared to the negative control group. In addition, a clinical score was given to the different mammary glands after infection. Both the cefalonium and phage-treated groups scored significantly better than the negative control group.

In vitro and in vivo evaluation of phage-derived endolysins in the context of bovine mastitis

In a study by Zhou et al. (2017), a recombinant, lytic enzyme (LysKΔamidase) was constructed out of a staphylococcal phage lysin, in which a broad lytic activity of LysKΔamidase was observed against 137 methicillin-resistant and methicillin-susceptible staphylococcal strains isolated from human hospital patients and cows with bovine mastitis. In addition, this lytic enzyme was also found to disrupt the normal structure of biofilms, which are protective structures produced by bacteria (i.e. *S. aureus*) that consist of DNA, proteins and carbohydrates. The in vitro potential of endolysins was demonstrated to combat MRSA and other antimicrobial-resistant, biofilm-forming staphylococcal strains associated with bovine mastitis.

In a study by Donovan et al. (2006), an endolysin derived from a *S. aureus* bacteriophage phi11 was purified and its effectiveness was demonstrated against *S. aureus* and non-aureus staphylococci. Its lytic activity was maintained at the pH (6.7) and Ca^{2+} concentration (3 mM) of milk, making phi11 endolysin a potential candidate as antimicrobial protein.

In a study by Fan et al. (2016), another recombinant endolysin (Trx-SA1) from a *S. aureus* bacteriophage was derived by cloning it into the pET-32a bacterial expression vector. Subsequently, an efficacy trial of its effectiveness against bovine mastitis was conducted. When Trx-SA1 was added to the host bacteria in early growth stages, a complete bacterial lysis was observed after nine hours. Preliminary results of a proof-of-concept therapeutic trial in cow udders showed that Trx-SA1 could effectively control mild clinical mastitis caused by *S. aureus*.

Phage-derived λSA2 and B30 endolysins were tested in vitro and in a mouse mastitis model against bovine streptococci (Schmelcher et al., 2015). Lytic activities were observed to be optimal at ionic strengths, pH, and Ca^{2+} concentration consistent with those in cow milk. Moreover, λSA2-endolysins were demonstrated to reduce in vitro the amount of *S. agalactiae*, *S. dysgalactiae* and *S. uberis* in cow milk by a log 2, 3.5, and 4 CFU/mL, respectively. Interestingly, although the B30 endolysin alone turned out to be less effective, a strong synergy appeared with the λSA2-endolysin. When further tested in a mouse model for bovine mastitis, a significant decrease in CFU was observed after intramammary inoculation of these endolysins in vivo.

CRITICAL COMMENTS AND FUTURE PROSPECTS

With only about ten promising in vitro and few in vivo studies reported till date, more research should be performed, especially in vivo, on the clinical applicability of either bacteriophages or their endolysins for the curative treatment of udder infections. Nevertheless, it may be summarized that most of these current experiments show the effectiveness -at least in vitro- of phage therapy against *S. aureus*. There is an urgent demand for alternative therapies against this Gram-positive mastitis pathogen, because staphylococcal intramammary infections are typically difficult to combat with classic antibiotics (Holmes et al., 2011). Persistence of this germ in the mammary gland results in chronic, subclinical mastitis. In addition, resistance against *S. aureus* is quickly established and persistent (Kadlec et al., 2012).

Recently, several researchers have claimed that promising candidate bacteriophages and endolysins should now be tested in vivo to evaluate the effects of this novel treatment strategy in mammary gland of rodents, but preferably in the target species i.e. the cow (Schmelcher et al., 2015; Porter et al., 2016). How-

ever, even if the envisaged phage therapy confirms to be promising in these follow-up studies, several practical hurdles will raise during its development. It is known that raw milk inhibits staphylococcal phage K proliferation due to the formation of bacterial clusters associated with fat globules and/or the presence of IgG (O'Flaherty et al., 2005; Tanji et al., 2015). In addition, Phage K has been reported to cause an increase in the SCC of healthy quarters (Gill et al., 2006). Most strains belonging to the group of Gram-positive mastitis pathogens are also known to cause biofilms, a property associated with their difficult eradication by traditional antimicrobial drugs. It should be remarked that biofilm-formation also occurs in some Gram-negative mastitis-causing bacteria such as *Klebsiella*. As mentioned above, phages and endolysins have been described to have the unique characteristic to digest these protective structures and are expected to be able to infect and lyse these problematic biofilm-forming, mastitis-causing bacteria (Latka et al., 2017; Zhou et al. 2017; Gerstmans et al. 2016; Gutiérrez et al., 2014).

Although some bacteria can be naturally resistant to bacteriophages due to the lack of required adhesion molecules on their bacterial cell wall, induced resistance has not yet been described. If resistance would nevertheless occur, supplementing the phage therapy with endolysins could be a useful tactic. Due to the broader spectrum of action, resistance selection among the pathogens against the used phage type may be expected to be counteracted. Still, it has been stated that phage therapy could give rise to antibiotic resistance as bacteriophage therapy is not capable of breaking down plasmids (Colavecchio et al., 2017). After lysis, these plasmids could easily be taken up by other bacteria. If they contain resistance genes, then this induced resistance may spread between the surviving bacteria (De Vliegher, 2017).

In addition, in the in vivo and some in vitro studies mentioned above, only a reduction of the bacterial count was observed, not a complete killing. This incomplete lysis of bacteria confirms the suboptimal effectiveness of current bacteriophages as well as phage-derived endolysins, even after enhancement of the latter by genetic engineering. Moreover, it should be noted that the delay time between experimental infection and administration of either bacteriophages or phage-derived endolysins in the used mouse mastitis model is very short, i.e. typically thirty minutes. Consequently, this set-up probably does not allow the mastitis pathogens to enter intracellularly nor to form biofilms. These experiments should be adapted to provide more relevant conditions for bovine mastitis as occurring on dairy farms.

A treatment consisting of only one bacteriophage can never provide a broad-spectrum effect due to the species specificity inherent to phages (Nilsson, 2014). In contrast, for endolysins, the spectrum can be enhanced through genetic engineering. Indeed, endolysins derived from bacteriophages that target Gram-positive bacteria feature a modular design, consisting of enzymatically active domains and cell wall binding domains. This modular architecture enables the creation of chimeric fusion proteins with novel enzymatic and antimicrobial properties. In two parallel recent studies by Becker et al. (2016) and Rodríguez-Rubio et al., (2016), an engineered staphylolytic and streptolytic fusion protein have been reported, respectively, with improved activities. Furthermore, the additional fusion to positively charged peptides significantly enhanced both the ability to kill intracellular mastitis pathogens and biofilm eradication, and reduced the incidence of resistant strain development against these engineered endolysins. It is therefore important to pursue research for more potent, novel bacteriophages and to genetically improve their derived endolysins, to obtain an effective and fast-acting engineered fusion endolysins with broad spectrum effect and a minimal induction of resistance.

If phage therapy consists of applying only one phage type, the treatment is targeted against one specific type of bacterium. For the application in veterinary practice however, it is more interesting to obtain a broader spectrum of activity. Bacteriological culture of milk samples from dairy cows is not done routinely and includes an additional cost for the dairy farmer. It may therefore be useful to develop cocktails consisting of different bacteriophages or to supplement the phage therapy with endolysins. The expansion of the working spectrum has already been tested and confirmed by two recent studies (Porter et al., 2016; Breyne et al., 2017). The main disadvantage of the classical endolysins remains their Gram-positive spectrum. Despite the lack of potential to kill Gram-negative germs, it should be emphasized that most of the problematic (intracellular and biofilm-forming) mastitis pathogens are indeed Gram-positive bacteria (Piepers et al., 2007). Moreover, as already demonstrated in human medicine, artificial modification of existing endolysins, so-called Artilysins® (Lysando AG, Germany), may provide a broader spectrum of activity. These Artilysins® specifically attack Gram-negative bacteria (Gerstmans et al., 2017). In addition, it has been demonstrated that artilysation of currently known endolysins also improves the lytic activity against Gram-positive germs (Rodríguez-Rubio et al., 2016).

From the point of view of the dairy practitioner, a broad spectrum, long-acting, intramammary preparation is needed if phage and/or endolysin therapy is envisaged to be used in the local treatment of bovine mastitis. The principal advantage gained from a novel phage/endolysin cocktail would be the reduced use of antibiotics, more specifically, those that are critical for human health. Residues in milk may then be avoided, implying that the dairy farmer does not have to discard milk derived from phage-treated animals as is now obligatory for antibiotic-treated animals. It can also be noted that the in vivo therapeutic concentration and the treatment interval are unknown fac-

tors that depend on the type of bacteriophages and/or endolysins used. A pharmacokinetic study was conducted, in which bacteriophage therapy was tested in subclinical *S. aureus* mastitis in lactating dairy cattle (Gill et al., 2006). Phage K persisted 36 hours within an infused quarter, but the effective concentration was significantly lower than predicted by simple dilution in produced milk. This implicates that more pharmacokinetic studies are also mandatory in the development of phage and/or endolysin-based mastitis drugs.

Finally, in contrast to human medicine, phage therapy has not yet been incorporated into the European legislation for veterinary medicinal products. As registered veterinary medication based either on bacteriophages or on endolysins does not yet exist, their current therapeutic use is considered as a magistral preparation. This implicates that their use is justified for the individual treatment of an animal to avoid unnecessary suffering, after prescription by a veterinarian and preparation by a pharmacist. From the point of view of veterinary practice, this is not desirable because the dairy farmer cannot start therapy immediately when detecting mastitis in one of his animals. Moreover, it does not make sense to apply individual mastitis therapy using a magistral preparation.

CONCLUSION

In conclusion, currently reported data clearly indicate that bacteriophages and phage-derived endolysins constitute a potential therapeutic alternative in the treatment of bovine mastitis. Nonetheless, these studies were predominantly carried out in a preclinical context. Further research should now evaluate whether this promising therapy is also active in the complex bovine mammary gland. If these results are confirmed, bacteriophages and phage-derived endolysins could indeed fulfil their promise by reducing 1. the excessive use of antibiotics in the dairy industry and 2. the common antimicrobial resistance in mastitis-causing pathogens. Furthermore, this alternative treatment would also become an important strategy to counteract the antimicrobial resistance in human pathogens as viewed from the 'One Health' perspective.

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Risk factors for antimicrobial use in food-producing animals: disease prevention and socio-economic factors as the main drivers?

Risicofactoren voor antibioticumgebruik bij voedselproducerende dieren: ziektepreventie en socio-economische factoren als drijfveer?

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ABSTRACT

The European Union requests an urgent decrease in antimicrobial use (AMU) in food producing-animals to reduce antimicrobial resistance in animals and humans and safeguard the efficacy of antimicrobials for future generations. The identification of risk factors (RFs) for AMU is essential to obtain a rapid reduction. The aim of this review was to summarize the current knowledge of RFs for AMU in veal calves, pigs and poultry. Thirty-three observational studies were included. Well-identified RFs for an increased AMU are frequent purchase of animals, herd size (large or small depending on the animal species), and a lack of selected biosecurity measures. Also in beef breed calves, more antimicrobials are used than in Holstein calves. AMU is influenced by the farmer, the veterinarian and by the integration. In general, socio-economic RFs are largely unexplored. The causal factors for AMU are multiple and complex, with possible confounding factors and unidentified interactions. Additional knowledge of socio-economic drivers appears particularly urgent to create tailor-made guidelines and awareness campaigns for each sector.

SAMENVATTING

De Europese Unie vraagt om een dringende reductie van het antimicrobieel gebruik bij voedselproducerende dieren. Het uiteindelijke doel is een daling van het antimicrobiële resistentieniveau bij mens en dier en de doeltreffendheid van antimicrobiële middelen te behouden voor toekomstige generaties. De identificatie van risicofactoren voor antimicrobieel gebruik is essentieel om deze reductie te behalen. Dit overzichtsartikel heeft als doel de huidige kennis omtrent risicofactoren voor antimicrobieel gebruik bij vleeskalveren, varkens en pluimvee samen te vatten. Drieëndertig observationele studies voldeden aan de selectiecriteria. Bekende risicofactoren van antimicrobieel gebruik zijn de frequente aankoop van dieren, de grootte van de kudde (groot of klein, afhankelijk van de diersoort) en de afwezigheid van bepaalde bioveiligheidsmaatregelen. Bij witvleeskalveren worden er bij de vleesrassen meer antimicrobiële middelen gebruikt dan bij holsteinkalveren. Het antimicrobiële gebruik wordt beïnvloed door zowel de veehouder, de dierenarts als de integratie. In het algemeen worden socio-economische risicofactoren onvoldoende onderzocht. De uitlokkende factoren van antimicrobiële gebruik zijn multipel en complex, met mogelijke "confounders" en (nog) niet-geïdentificeerde interacties. Bijkomende kennis van de socio-economische factoren is cruciaal voor het ontwerpen van sectorspecifieke richtlijnen en sensibiliseringscampagnes.

INTRODUCTION

Antimicrobial resistance (AMR) is a worldwide health problem in humans and animals (EU, 2016; EFSA, 2017). It causes therapy failure with prolonged hospitalization, increased antimicrobial use (AMU) and mortality risk (Watts and Sweeney, 2010; Economou and Gousia, 2015). If no measures are taken, by 2050, ten million people per year might possibly die of AMR (O'Neill, 2016). Resistant bacteria and their genes can transfer between animal and human hosts directly or indirectly by food intake or through the environment (Box et al., 2005; Bosman et al., 2014). This is the most important reason why the Council of the European Union is determined to approach this health issue from a 'One Health' perspective, demanding collaboration and mutual efforts from both the human health sector as the agricultural and veterinary sectors (EU, 2016).

Food-producing animals, especially those reared under intensive conditions, like veal calves (Graveland et al., 2011; Haenni et al., 2014), pigs (Smith et al., 2009; Mutters et al., 2016) and poultry (Mulders et al., 2010; Persoons et al., 2011; Kluytmans et al., 2013), are important reservoirs for AMR genes (Callens et al., 2017). These industries have in common the use of both group and oral antimicrobial treatments (Casal et al., 2007; Callens et al., 2012; Pardon et al., 2012a; Persoons et al., 2012; Arnold et al., 2016), which are highly associated with AMR (Dunlop et al., 1998; Varga et al., 2009). However, every use of antimicrobials selects for AMR (Barbosa and Levy, 2000), which is seen in pathogens but also in commensal bacteria and zoonotic agents. In addition, the transfer of multidrug resistant bacteria between animals and humans is worrisome, for example methicillin-resistant *Staphylococcus aureus* (MRSA) in pigs and veal calves (Smith et al., 2009; Graveland et al., 2010), the emergence of extended spectrum beta-lactamase-producing *Enterobacteriaceae* (ESBL) in veal calves and poultry (Hordijk et al., 2013; Kluytmans et al., 2013) and the recent discovery of transferable colistin resistance in *Escherichia coli* from veal calves (Malhotra-Kumar et al., 2016), pigs (Brauer et al., 2016; Liu et al., 2016), poultry (MARAN, 2016) and humans (McGann et al., 2016). AMR in animals is monitored in different countries in foodborne pathogens *Salmonella enterica* and *Campylobacter* spp. and in commensal indicator bacteria, such as *Escherichia coli* for Gram-negative bacteria and *Enterococcus faecium* and *Enterococcus faecalis* for Gram-positive bacteria (EFSA, 2017). Of all food-producing animals, veal calves, pigs and poultry have high (multi)resistance levels, in contrast to dairy and beef cattle (Kaesbohrer et al., 2012; Chantziaris et al., 2014; Hanon et al., 2015; CODA, 2016; Dorado-García et al., 2016).

The most important risk factor (RF) for developing AMR is AMU (Barbosa and Levy, 2000; Bosman et al., 2013; Holmes et al., 2016). Therefore, the reduction of AMU is a top priority in the global health policy (WHO, 2011). In several EU countries, like Belgium, a new legislation has been initiated requiring sampling and antimicrobial sensitivity testing before critically important antimicrobials (in casu fluoroquinolones and third- and fourth-generation cephalosporins) can be used (KB 21st of July, 2016). Also, benchmarking farmers and veterinarians is done in different EU countries (www.aacting.org). This system allows farmers to compare their usage at the farm and veterinarians to compare prescription behavior with each other. Independent or governmental organisations are then able to identify high users and may stimulate them towards a reduced use or less antimicrobial-based prescriptions (SDa, 2016). Between countries, whether their general use is high or low, there is a huge variation in antimicrobial usage between farms and sectors (Pardon et al., 2012a; Bos et al., 2013; Sjölund et al., 2016). To be able to rationally reduce antimicrobial consumption, knowledge of the drivers of AMU is essential. Therefore, the objective of the present review was to summarize currently identified RFs for AMU in food-producing animals (veal calves, pigs and poultry).

MATERIALS AND METHODS

A search was conducted in Pubmed, Web of Science and Google Scholar on the following terms and their combinations: calves, pigs, poultry, cattle, antimicrobial use, antibiotic use, risk factor and socio-economics. Primary inclusion criteria were an observational study design and the use of standard daily dose methodology to quantify on-farm AMU (Jensen et al., 2004).

RISK FACTORS FOR ANTIMICROBIAL USE

The literature search identified a total of twenty articles with the primary inclusion criteria. Six studies for veal calves, twelve studies for pigs, and two studies for poultry, which in total identified 27 different RFs for AMU. Nine articles were excluded because of inadequate compliance with the STROBE guidelines (Elm et al., 2014). An overview of the significant RFs in veal calves, pigs and poultry is provided in Table 1. Most studies used 'defined daily doses' for animals (DDDvet), three used 'used daily dose' (UDD) and only one 'prescribed daily dose' (PDD). In the next paragraphs, an overview of the identified RFs for AMU is provided. RFs can be divided in two large groups, namely those associated with disease and/or disease prevention and those associated with socio-economic drivers. The interaction between these RFs is complex and extensive schematic representations are available elsewhere (Lhermie et al., 2016). A sim-

plified version is presented in Figure 1. The figure contains the different groups of RFs identified. The level of evidence for these RF groups is only derived from observational studies, as no randomized clinical trials or experimental studies were available to establish causality. The reporting of non-evidenced (hypothetical) relationships of RF groups with AMU was limited to these groups, judged as essential to provide an overview of what is important, based on human studies, but currently unexplored in food-producing animals. In Figure 1, the farmer's decision to use antimicrobials is put centrally in the causal diagram. Presenting the decision to use antimicrobials as a joint decision between farmer and veterinarian would likely most correctly represent the current situation in the field. However, more studies are needed to support this theory.

Risk factors for antimicrobial use associated with disease and/or disease prevention

From a perspective of rational AMU, disease associated with bacterial infection should be the primary motivator for AMU (Figure 1). Unfortunately, the most recognized RF for AMU is disease prevention (Chauvin et al., 2005; Casal et al., 2007; Pardon et al., 2012a; Arnold et al., 2016; Jarrige et al., 2017). In veal calves in France for example, 'starting treatments', i.e. treatments received in the first 15 days of fattening, are responsible for 33.7% of the AMU (Jarrige et al., 2017). In pigs, 58% (Casal et al., 2007) up to 93% (Arnold et al., 2016) of the antimicrobials are used as a prophylactic oral therapy. Only a small percentage (7%) of the antimicrobials in pigs are used after diagnosis with pneumonia, diarrhea and lameness (Arnold et al., 2016). In contrast, only a couple of studies do associate the presence of disease with AMU (Hughes et al., 2008; Sjölund et al., 2015; Lava et al., 2016b). Lava et al. (2016b) showed that a 10% increase in bovine respiratory disease (BRD) incidence is a RF for metaphylactic antimicrobial therapy in veal calf farms. BRD is the main indication for AMU in veal calves, accounting for 53% of group treatments and up to 79% of the total AMU (Sargeant et al., 1994; Pardon et al., 2012; Lava et al., 2016a; Fertner et al., 2016). The relationship between disease and AMU is further supported by the observation that specific pathogen free (SPF) Swedish farrow-to-finish pig herds use significantly less antimicrobials compared to non-SPF herds (Sjölund et al., 2015). In poultry, positive associations between necrotic enteritis, coccidiosis, feet disorders and respiratory diseases and AMU have been demonstrated (Hughes et al., 2008; Persoons et al., 2012). It is important to realize that in intensively reared, food-producing animals, disease frequency estimates have historically been often blurred by the preventive/metaphylactic antimicrobial treatments on arrival. For example, in veal calves, 13 to 34% of the total AMU accounts for treatment on arrival (Pardon et al., 2012a; Jarrige et al., 2017).

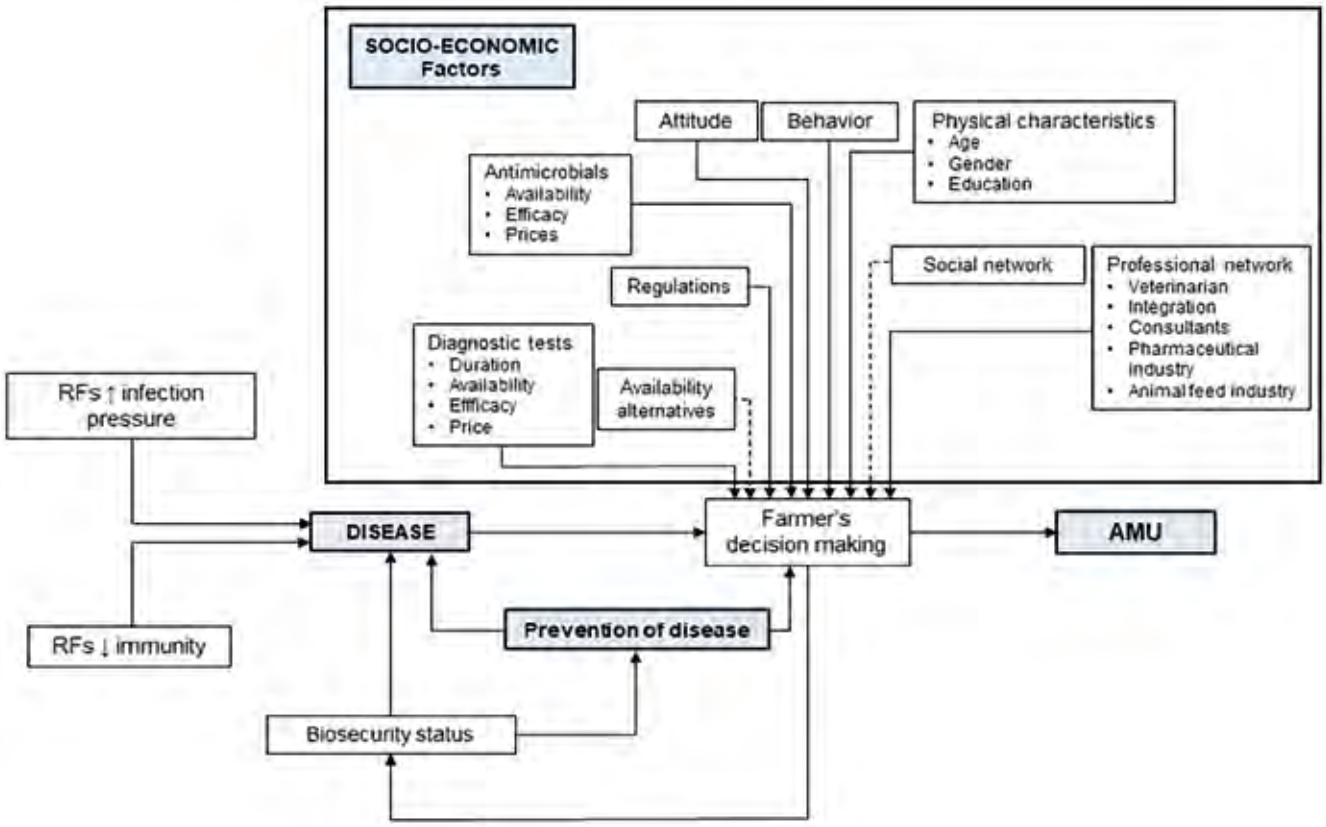

Figure 1. Causal diagram illustrating epidemiologically evidenced (full line) and hypothetical (dotted line) associations between groups of risk factors (RFs) and AMU in food animals (veal calves, pigs and poultry).

Next, identified RFs for AMU, which are associated with disease and disease prevention, are summarized. RFs that increase infection pressure or pathogen spread may be distinguished from RFs that compromise immunity.

Increased infection pressure

Purchase and size of the herd

Purchase is a major RF for AMU identified in veal calves and pigs (Casal et al., 2007; Hybschmann et al., 2011; Van der Fels-Klerx et al., 2011; Fertner et al., 2016; Lava et al., 2016b). Purchase is still most prominently present in the veal industry, but whether calves originate from a market or directly come from the farm of origin does not affect the total AMU (Fertner et al., 2016). Commingling piglets from different farms is a higher risk for oral AMU than the purchase from a single farm (Arnold et al., 2016). Hughes et al. (2008) reported that when broilers were purchased from different hatcheries, the therapeutic AMU reduced, but preventative AMU increased. No information about the total AMU was shown. In another study however, it was concluded that prophylactic use in turkeys is associated with a higher AMU (Chauvin et al., 2005). In contrast, in a study with veal calves, therapeutic antimicrobial treatment of BRD was higher in herds not receiving arrival treatment. However, the total AMU over the study period was the same in both groups (Rérat et al., 2012).

Purchase and commingling likely influence AMU through a higher disease incidence. When commingling, animals are exposed to an increased number of pathogens (Callan and Garry, 2002) and to stress caused by transport and creating new groups (Carroll and Forsberg, 2007), leading to increased morbidity rates and subsequent AMU. In a veal setting, an increased herd size is always linked with a higher degree of purchase and more herds of origin. In a study with Swiss veal calves, the likelihood to administer metaphylactic antimicrobial therapy increased significantly with a larger herd size, more farms of origin and a higher number of calves per pen (Lava et al., 2016b). In pigs, there is a contradiction of the effect of herd size on AMU. Several studies have shown an increased AMU with an increased number of sows on the farm (Van der Fels-Klerx et al., 2011; Backhans et al., 2016; Temtem et al., 2016). It is possible that disease was a confounding/intervening factor in these studies, as herd size is also an identified RF for developing different diseases in pigs (Tuovinen et al., 1992; Maes et al., 2001), because of the increased risk of introduction and pathogen spread in larger herds. In contrast, Hybschmann et al. (2011) found a negative association between herd size and AMU for gastrointestinal diseases. This is in line with Vieira et al. (2011), who studied fattening pigs and also concluded that smaller herds are a RF for AMU. In another study, an influence of farm size on AMU was found, but only when accounting for the veterinarian (van Rennings et al., 2015). Postma et al. (2016) did not find a link between herd size and AMU in a study on 227 pig herds. A possible explanation is that some veterinarians deal with farms with different sizes and treatment protocols may be highly variable. Moreover, it might be that larger herds are managed in a more professional way, with a higher level of biosecurity, less pathogen spread and less disease (Gardner et al., 2002; Van der Wolf et al., 2011; Laanen et al., 2013). So far, in poultry, flock size as a RF for AMU has not been identified yet. However, a larger flock is positively associated with disease (Tablante et al., 2002) and mortality (Heier et al., 1999).

Altogether, purchase is a well-identified RF, whereas herd size is less clear, because of the additional differences, which are associated with herd size, i.e. purchase, herds of origin, infection pressure, management and biosecurity.

Internal and external biosecurity

Biosecurity can be separated in internal and external biosecurity. External biosecurity is about keeping pathogens from entering a herd (Laanen et al., 2013) and internal biosecurity deals with reducing infection pressure within a herd. Laanen et al. (2013) found a negative association between biosecurity scores and prophylactic AMU in breeder-finisher pig herds in Belgium, indicating that higher biosecurity scores are associated with lower AMU levels. They divided measurements in internal biosecurity, i.e. disease management, different units, cleaning and disinfection, and external, i.e. purchase, transport and environment, and combined these to an overall score. The overall score and the internal biosecurity were both negatively associated with AMU, whereas there was no relationship with external biosecurity. In contrast, a Swedish study on farrow-to-finish farms showed no association between biosecurity and AMU at all (Backhans et al., 2016). Possible explanations are the already very low AMU and the advanced internal biosecurity in Swedish farms (Postma et al., 2016a), and an overall better health status of the pigs (free of porcine reproductive and respiratory syndrome virus) or a lack of power in this study. The strongest associations reported by Laanen et al. (2013) were disease management and measurements during the birth and suckling period. In another study, treating ill animals before visiting the healthy piglets and the absence of an all-in-all-out production system were RFs for oral AMU (Arnold et al., 2016). Considering external biosecurity, the use of quarantine and performing a clinical examination upon arrival have been associated with a lower AMU in Swiss veal calves (Lava et al., 2016a). In pig farms, the availability of changing facilities has been associated with lower prophylactic AMU (Casal et al., 2007) and the absence of working clothing has

been a RF for oral AMU (Arnold et al., 2016). In turkey, changing clothes and shoes before entering the facility has also been associated with lower AMU (Chauvin et al., 2005). Farmers working in a single farm are also a RF for increased AMU, probably because there is less exchange of knowledge about biosecurity (Arnold et al., 2016). Farms with a higher external biosecurity status have been associated with a lower AMU (Postma et al., 2016a).

Hygiene is an internal biosecurity factor, which influences AMU. Poor hygiene of the water supply system has been associated with an increased oral AMU in recently weaned piglets (Hirsiger et al., 2015). Similarly, also at broiler farms not controlling water quality has been a RF for increased AMU (Persoons et al., 2010). In veal calves, the effect of hygiene has hardly been studied, but disinfection between batches (Jarrige et al., 2017) and cleaning frequency within or longer than thirty days have not been associated with AMU (Lava et al., 2016a). In broiler farms, wet litter is a RF for therapeutic AMU (Hughes et al., 2008). In this association, disease is probably a confounder, because wet litter is often a result of coccidiosis (Hermans et al., 2006) and may induce ulcerative lesions resulting in secondary infections (Martland, 1985).

In summary, the relationship between biosecurity measures and AMU in the different sectors is complex and likely severely influenced by behavioral factors/farmer characteristics (Backhans et al., 2016) and the presence of particular pathogens in a given farm. It is important to realize that so-called 'early adapters', might take efforts to reduce AMU and increase biosecurity at the same time to comply with current societal demands from the industry.

Housing and region

In only five studies, the relationship between housing factors and AMU has been explored. Lava et al. (2016a) concluded that a shared air space by different groups of white veal calves is positively associated with AMU. Additionally, housing pigs with age differences larger than one month in a shared air space is a RF for respiratory diseases (Jäger et al., 2012), which may indicate that disease is the direct driver for the higher AMU. Moreover, Jarrige et al. (2017) concluded that calves housed with six to ten animals in a pen are more treated with antimicrobials than pair-housed calves. Also, separated feed and lying area are positively associated with AMU (Lava et al., 2016a). Influence of ventilation system, floor type, number of calves per nipple and stall climate on AMU has not been reported (Lava et al., 2016a; Jarrige et al., 2017).

Additionally, regional farm density affects AMU. Oral AMU is higher when more sow-farms are present (Van der Fels-Klerx et al., 2011) or when the next pig farm is located within 500 metres (Arnold et al., 2016). Also Hybschmann et al. (2011) found an association between region and AMU in pigs with gastrointestinal problems, and Lava et al. (2016a) found a regional effect on AMU in veal calves. Other studies in veal calves (Jarrige et al., 2017) and pigs (van Rennings et al., 2015) could not identify any regional effects. Disease incidence and likely also the treating veterinarian and the socio-economic background of the region may act as confounders for these regional differences and housing effects. For example, swine density in an area is a known RF for seroprevalency of different pathogens (Maes et al., 2000).

Housing (shared airspace, pen density and separated feed and lying area) and regional farm density influence AMU; however, further research is needed because only a few factors associated with housing and region have been investigated.

Year and season

A significant annual variation in AMU has been documented in Belgian veal calves (Bokma, 2017). A possible reason may be the variable meteorological conditions, i.e. temperature, humidity and abrupt changes, which affect the infection pressure and exposure to cold stress. In the same study by Bokma (2017), independent of year, calves which arrived in the warmer months of the year, e.g. May, were administered significantly less antimicrobials than calves arriving in September to December. Also in Danish veal calves, the largest AMU has been seen in autumn and winter (Fertner et al., 2016). Other explanations for an annual variation in AMU might be influences of legislation and campaigns concerning antimicrobial reduction or other currently unidentified socio-economic drivers.

Mortality

Results from a study in white veal calves in France showed that more antimicrobials were used in farms with mortality risks over 5% (Jarrige et al., 2017). Casal et al. (2007) found a lower frequency of prophylactic AMU when the mortality rate was beneath 3% in pigs. In broilers, a higher mortality rate has been associated with increased therapeutic AMU (Hughes et al., 2008). Until today, the risk of an increased mortality when lowering AMU, as feared by all food-animal sectors, has actually not been substantiated by any study.

Compromised immunity

Apparently, 77% of Dutch veterinarians and 67% of Flemish veterinarians (n=611 veterinarians) believe a compromised immune system is an important reason for AMU (Postma et al., 2016b). However, hard evidence on the association of compromised immunity and the need for AMU is completely lacking. In calves, breed has been associated with an increased AMU on different occasions, with beef breeds, in which more

Table 1. Overview of identified risk factors (RFs)* for antimicrobial use (AMU) in food-animal studies.

Veal calves		Pigs		Poultry	
Reference	**Identified RFs**	**Reference**	**Identified RFs**	**Reference**	**Identified RFs**
Bokma (2017)	Belgium blue breed	Arnold et al. (2016)	No work sequence depending on healthy before sick pigs	Chauvin et al. (2005)	One full-time job at farm
	Integration		Working on other farms		No changing of clothes and shoes upon entering the facilities
	Month of arrival		Distance to the next pig farm < 500m		No competitive exclusion flora administration
	Year		Absence of visitor boots		Prophylactic antimicrobial treatment
Fertner et al. (2016)	Number of calves introduced		No analysis of production parameters	Persoons et al. (2010)	No control of water quality
	Season		No application of homeopathic agents		Bad hygienic condition of medicinal treatment reservoir
Jarrige et al. (2017)	Number of calves per pen		Mixing pigs of different suppliers within the same pen		
	Mortality rate	Backhans et al. (2016)	Number of sows		
Lava et al. (2016a)	Beef breed		Gender farmer		
	No clinical examination upon arrival		Education farmer		
	No quarantine upon arrival		Age farmer		
	Same air space different groups	Callens et al. (2012)	Weaned piglets		
Pardon et al. (2012a)	Smaller integration size	Hirsiger et al. (2015)	Poor hygiene of water supply		
			< 2 veterinary visits per year		
			No analysis of production parameters		
			Continuous occupation		
		Kruse et al. (2016)	Vaccination against PCV-2		
			Vaccination against *M. hyopneumoniae*		
		Laanen et al. (2013)	Disease management		
			Farrowing and suckling period		
			Inadequate biosecurity		
		Postma et al. (2016a)	Weaned piglets		
			Vaccination		
			Inadequate biosecurity		
		Sjölund et al. (2015)	No specific pathogen free herd		
		Sjölund et al. (2016)	Weaned piglets		
		Temtem et al. (2016)	Number of sows		
			Vaccination against PCV-2		
			Vaccination against *M. hyopneumoniae*		
		Van der Fels-Klerx et al. (2011)	Farm system		
			Population density of region		
			Number of sows		
		Van Rennings et al. (2015)	Farm size		
			Weaned piglets		

*all mentioned risk factors are positively associated with AMU (increased usage)

Table 2. Overview of studies on socio-economic drivers for antimicrobial use (AMU) included in the present review.

Reference	Year	Title
Cattaneo et al.	2009	Bovine veterinarians' knowledge, beliefs, and practices regarding antibiotic resistance on Ohio dairy farms
De Briyne et al.	2016	Factors influencing antibiotic prescribing habits and use of sensitivity testing amongst veterinarians in Europe
Ge et al.	2014	A Bayesian Belief Network to infer incentive mechanisms to reduce antibiotic use in livestock production
Gibbons et al.	2013	Influences on antimicrobial prescribing behaviour of veterinary practitioners in cattle practice in Ireland
Jones et al.	2015	Factors affecting dairy farmers' attitudes towards antimicrobial medicine usage in cattle in England and Wales
McDougall et al.	2016	Factors influencing antimicrobial prescribing by veterinarians and usage by dairy farmers in New Zealand
Postma et al.	2016b	Opinions of veterinarians on antimicrobial use in farm animals in Flanders and the Netherlands
Speksnijder et al.	2014	Determinants associated with veterinary antimicrobial prescribing in farm animals in the Netherlands: a qualitative study
Speksnijder et al.	2015	Attitudes and perceptions of Dutch veterinarians on their role in the reduction of antimicrobial use in farm animals
Stevens et al.	2007	Characteristics of commercial pig farms in Great Britain and their use of antimicrobials
Visschers et al.	2014	Swiss pig farmers' perception and usage of antibiotics during the fattening period
Visschers et al.	2015	Perceptions of antimicrobial usage, antimicrobial resistance and policy measures to reduce antimicrobial usage in convenient samples of Belgian, French, German, Swedish and Swiss pig farmers
Visschers et al.	2016	A comparison of pig Farmers' and veterinarians' perceptions and intentions to reduce antimicrobial usage in six European countries

antimicrobials are used than in dairy breeds (Lava et al., 2016a; Bokma, 2017). For the Belgian blue beef breed, this can possibly be explained by a difference in susceptibility of respiratory diseases, due to their anatomy (Bureau et al., 1999; Pardon et al., 2012b) or socio-economic drivers, like risk aversion (Bokma, 2017). Also young age is believed to increase disease susceptibility and subsequently AMU, but studies have shown different outcomes. In veal calves, Bähler et al. (2016) did not find an association between age at introduction at the farm and AMU. In pigs, weaned piglets have shown the highest AMU (Callens et al., 2012; Postma et al., 2016a; Van Rennings et al., 2015; Sjölund et al., 2016). In contrast, Stevens et al. (2007) did not find any age effect in pigs, which is possibly due to general herd health in this study.

To improve immunity, a lot is expected from vaccination as a tool to reduce AMU. Unfortunately, the amount of peer-reviewed studies on this matter is limited. In veal calves, both Fertner et al. (2016) and Jarrige et al. (2017) did not find any effect of vaccination against BRD on AMU. Also in pigs, there has been no association between vaccination against *Lawsonia intracellularis* (Sjölund et al., 2015; Kruse et al., 2016; Temtem et al., 2016) or *Mycoplasma hyopneumoniae* (Sjölund et al., 2015) and AMU. Moreover, in Great Britain, vaccinating suckling piglets and weaners has been significantly associated with an increased AMU in feed (Stevens et al., 2007). Vaccinating weaners against porcine circovirus type 2 (PCV-2) and *M. hyopneumoniae* and vaccinating broilers against infectious bursal disease (IBD) has led to an increased AMU (Hughes et al., 2008; Kruse et al., 2016; Temtem et al., 2016). In pigs, Postma et al. (2016a) found a positive association between the number of pathogens vaccinated against and AMU, suggesting that in farms where more vaccines are used, also more antimicrobials are used. A possible explanation might be that in herds and flocks facing a high disease incidence, it might be more likely to start vaccinating next to continuing AMU to counteract the problem until the infection pressure is reduced (Postma et al., 2016a). To date, there is no clear evidence that vaccination reduces AMU; however, it is questionable if cross-sectional studies are fit to explore this topic.

In poultry, in only a handful of studies, nutritional influences on AMU have been looked at. In broilers, diets predisposing for necrotic enteritis, like whole wheat diets, have been associated with an increased AMU (Hughes et al., 2008). In contrast, controlled feeding regimes decrease preventive AMU (Hughes et al., 2008), possibly due to less foot lesion problems and reduced mortality rate (Robinson et al., 1992).

AMU due to decreased immunity may be influenced by breed and nutrition. Also age and vaccination may be a RF, but further research is needed (Figure 1).

Socio-economic drivers for AMU

Socio-economics drivers are factors based on how economic activity and social processes influence each other. In few studies, socio-economic RFs for AMU have been identified, and in only a few of them, standard daily dose methodology was used. Therefore, also studies dealing with socio-economic RFs for AMU, but not applying standard daily dose methodo-

logy, were included in this article. In Table 2, an overview of these thirteen studies is given.

Integrations, farmers and veterinarians

Next to RFs associated with disease, socio-economic drivers for AMU in food animals can be identified and are also shown in Figure 1. These socio-economic drivers influence the behavior of farmers, veterinarians and integrations. Behavior is an important influencer of the management in a farm, which subsequently affects both disease incidence and antimicrobial drug administration. An integration is a company that has ownership of different branches in the industry like transport, farms and slaughterhouses. Integration is very common in intensive foodanimal production (veal calves, pigs and poultry). In veal calves, an integration directly affects the amount of antimicrobials used in a particular farm (Pardon et al., 2012a; Bokma, 2017). Smaller integrations in Belgium are likely to use more antimicrobials for group treatments than larger integrations (Pardon et al., 2012a). More recently, a significant effect of the integration on the total AMU and on the use of critically important antimicrobials has been found (Bokma, 2017). In that research however, only one veterinary practice was studied, which excluded the veterinarian as a confounder. In contrast, Jarrige et al. (2017) found a smaller intraclass correlation coefficient (0.06) between integrations in France, compared to farmers (0.14) and veterinarians (0.12), indicating a smaller influence of integration than the influence of farmers and veterinarians in France.

Logically, the prescription behavior of a veterinarian has an effect on quantitative and qualitative AMU on farms in his/her practice. Although studies on characteristics of the veterinarian associated with his/her prescription behavior in food animals are lacking, it has been observed that older veterinarians worry less about AMR than their younger colleagues (Cattaneo et al., 2009; Speksnijder et al., 2015; McDougall et al., 2016). A reason could be that older veterinarians have not gotten the most recent education to create awareness on this topic in combination with preventive veterinary medicine.

In farmers, a positive association between risk aversion and prophylactic AMU has been identified (Ge et al., 2014). This could be explained by fear for disease. At least in some cases, a part of the prophylactic AMU is replaced by other products, like pro- or prebiotics, homeopathy or herbs. Arnold et al. (2016) identified homeopathic substances as a factor reducing AMU in pigs. This is in contrast to what Lava et al. (2016a) concluded in veal calves, namely that homeopathic therapy is not associated with AMU. Chauvin et al. (2005) and Hughes et al. (2008) concluded that the use of competitive flora is negatively associated with AMU. Competitive flora interferes with certain pathogens in the gastrointestinal tract and prevent diseases. When antimicrobials are used, this effect is probably nullified (Hakkinen and Schneitz, 1999). The previous findings can be explained by the risk-aversive nature of farmers or veterinarians who might just desire that the animals receive at least something to protect them; so, any replacement of antimicrobials will do (Arnold et al., 2016).

Also other socio-economic and management related RFs for AMU were identified in weaned piglets, i.e. less than two mandatory visits by the veterinarian a year (Hirsiger et al., 2015) and the absence of an internal analysis of production parameters (Hirsiger et al., 2015; Arnold et al., 2016). Also Visschers et al. (2014) showed that only using antimicrobials after asking a veterinarian is associated with a lower animal treatment index. These factors might refer to a less developed relationship between farmer and veterinarian, which is positively associated with AMU in pig farms (Visschers et al., 2016), suggesting the influence of socio-economic drivers upon management decisions.

Awareness of antimicrobial use

Next to studies directly on AMU data, there are some studies available focusing on the opinion of vets and farmers on AMU and AMR. The main reasons for farmers to use antimicrobials appear to be personal experience and veterinary advice (McDougall et al., 2016). However, veterinarians do think that the farmer's state of mind is one of the important reasons why antimicrobial consumption is that high in food animals (Postma et al., 2016b). In a recent study among pig farmers, it has been shown that it is not biosecurity measures, nor the attitude towards the use of antimicrobials, which determine AMU, but rather farmer's characteristics, such as age (higher use of antimicrobials when older), gender (more in females) and level of education of the farmer (more antimicrobial use when university education) (Backhans et al., 2016). However, this is in contrast with findings of Visschers et al. (2014), who did not find any relation between characteristics (age, years of experience) of the farmer and AMU.

Factors influencing the prescription of antimicrobials considered important by veterinarians are diagnosis, previous experience (Gibbons et al., 2013; McDougall et al., 2016; Postma et al., 2016b) and results from antibiograms (De Briyne et al., 2016; Postma et al., 2016b). Also non-clinical factors, such as withdrawal period (Speksnijder et al., 2014; McDougall et al., 2016), preferences and pressure from the farmer, price, temper of the animal, skills of the farmer (Gibbons et al., 2013), treatment interval and application route (Speksnijder et al., 2014) are important. In a study by Lava et al. (2016b), it was demonstrated that individual therapy reduces AMU in Swiss veal calves, but is sometimes difficult due to the temper of the animal and skills of the farmer. In contrast, Bokma (2017) found a positive association between a larger individual AMU and the total AMU, possibly because of frequently used long acting macrolides in that

study. In addition, risk management, such as fear to be blamed by the farmer afterwards and reducing animal suffering (Speksnijder et al., 2014), are important drivers for veterinarians to prescribe antimicrobials.

It still appears to be an important task to make farmers aware of the risk of AMR by excessive AMU (Visschers et al., 2014; Visschers et al., 2016). Of pig- and dairy farmers from New Zealand, England and Wales, respectively 26%, 30% and 32% are not aware of these risks (Stevens et al., 2007; Jones et al., 2015; McDougall et al., 2016). Moreover, there is a difference between countries. Especially French and Belgian farmers do not worry much about AMR in contrast to German, Swiss and Swedish farmers. Additionally, it is remarkable that Flemish pig farmers report to receive less information from their veterinarians about rational AMU, risks of AMU and alternatives for AMU than in other countries (Visschers et al., 2015).

Regulations and price-related objectives could help to reduce AMU (Ge et al., 2014; Visschers et al., 2014). By rising antibiotic costs, farmers with high AMU are more affected than those consuming less antimicrobial products. When farmers and veterinarians are asked about drivers, which will lead to reduce their AMU, farmers believe approval of their social network (Jones et al., 2015), cuts in meat price when pigs are treated with a lot of antimicrobials (Visschers et al., 2015), using vaccines and improving housing (Stevens et al., 2007) will reduce AMU. Motivational drivers for farmers to change their behavior are associated with animal welfare, economy (Visschers et al., 2015) and experience with therapeutic failure due to AMR (Visschers et al., 2016). Dutch veterinarians especially believe in the effect of benchmarking, improving feed quality (Speksnijder et al., 2015; Postma et al., 2016b) and housing (Speksnijder et al., 2015). In the Netherlands, benchmarking has already contributed to a noteworthy reduction in AMU, because veterinarians and farmers are able to compare themselves with colleagues (SDa, 2016). It confronts them with their own AMU, which leads to more awareness. More studies show that benchmarking will stimulate veterinarians and farmers to meet the regulations (Ge et al., 2014; Visschers et al., 2014; Visschers et al., 2016). Factors which keep farmers and veterinarians from reducing AMU, are in case of Dutch and Flemish farmers a financial matter (Visschers et al., 2015; Postma et al., 2016b). Reasons why they do not follow their veterinarians' advices is because of costs, too much time consuming measurements and contradictions in advices from different consultants at their farm (Speksnijder et al., 2014). It is important to mention the differences between countries in perception and behavior concerning AMU, which may demand different approaches to reduce AMU in different countries (Postma et al., 2016b; Visschers et al., 2016).

As mentioned earlier, studies directly evidencing the effect of behaviors on AMU are currently lacking. To alter behavior and habits to reduce AMU, it is necessary to change the motivation of farmers, veterinarians and integrators to use antimicrobials. These changes may be initiated by collecting knowledge on the key drivers of AMU and by changing the current attitude towards AMU (Trepka et al., 2001). It is highly recommended that also studies on socio-economic and behavioral drivers use standard daily dose methodology to express AMU, so comparability between international studies can be strengthened.

CONCLUSION

Despite the high pressure to reduce AMU in food-producing animals, to date, only few RFs for AMU have been identified in a limited number of studies, mostly in veal calves and pigs. RFs for AMU are multiple and complex, with many suspected interactions. A general stimulation of the different intensive food-animal industries towards less purchase and/or a better control of the infectious status of purchased animals are recommended. Improving biosecurity is preferentially done in a tailor-made manner, adapted to a specific farm situation to minimize the cost/benefit ratio. More clarity is needed whether the observed breed differences in AMU in veal calves reflect an increased disease susceptibility in beef breeds or are due to farmer's or veterinarian's risk aversion in the more expensive beef veal calves. The exact influence of housing, region or season needs more clarification in each industry before recommendations can be made. Next to disease and its prevention, the farmer's and veterinarian's decision making process is a key driver of AMU. The socio-economic drivers of this decision are currently almost unexplored in food animals, although knowledge of these factors is crucial to achieve behavioral changes through sector-specific guidelines and awareness campaigns.

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Diagnosis and treatment of lumbosacral vertebral instability caused by discospondylitis in a dog

De diagnose en behandeling van instabiliteit van de lumbosacrale wervelkolom ten gevolge van discospondylitis bij een hond

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ABSTRACT

A ten-month-old, female canine crossbreed of 40 kg was referred for episodes of severe pain non-responsive to analgesics. Neurological examination revealed pain on palpation of the lumbosacral region and non-weight bearing pelvic limb lameness, which was attributed to pain. Discospondylitis at L7-S1 was diagnosed based on radiographs and magnetic resonance imaging. Blood and urine culture were positive for *Staphylococcus* spp.. Despite ten days of conservative treatment, the dog did not show any improvement. Dynamic radiographs revealed a vertebral subluxation of L7-S1 in flexion. Surgery consisting of a dorsal laminectomy and stabilization of L7-S1 was performed. This resulted in a fast and complete recovery. Follow-up radiographs at six and twelve months after diagnosis showed severe osteolytic changes affecting L7. Despite these findings, the dog remained clinically normal.

SAMENVATTING

Een tien maanden oude, vrouwelijke hond van 40 kg werd doorverwezen omwille van erge pijn die niet verbeterde met pijnmedicatie. Neurologisch onderzoek toonde rugpijn aan in de lumbosacrale regio. De hond kon niet meer steunen op beide achterpoten door uitgesproken pijn. Met behulp van radiografieën en magnetic resonance imaging werd de diagnose van lumbosacrale discospondylitis gesteld. Het bloed- en urine-onderzoek was positief voor *Staphylococcus* spp.. De hond werd gedurende twee weken conservatief behandeld maar dit gaf geen beterschap. Dynamische radiografieën toonden een subluxatie in flexie van S1 ten opzichte van L7 naar ventraal. Er werd een dorsale laminectomie uitgevoerd gevolgd door stabilisatie van L7-S1. Dit resulteerde in een snel en volledig herstel. Radiografieën die genomen werden zes en twaalf maanden postoperatief toonden de aanwezigheid van uitgesproken osteolyse van L7 aan. Ondanks deze bevinding vertoonde de hond geen klinische symptomen.

INTRODUCTION

Discospondylitis is a primary infection of the cartilaginous vertebral endplates with secondary involvement of the intervertebral disc (Thomas, 2000; Burkert et al., 2005). Large, male, older and purebred dogs are predisposed (Thomas, 2000; Burkert et al., 2005). Less commonly, smaller dogs and cats are affected (Burkert et al., 2005; Packer et al., 2005). The most commonly affected site is the L7-S1 segment, followed by the thoracolumbar and cervical vertebral column (Burkert et al., 2005). In 40% of dogs diagnosed with discospondylitis, multiple sites are affected (Burkert et al., 2005). *Staphylococcus* spp. is the most common recognized infectious agent (Kornegay, 1986; Gilmore, 1987; Burkert et al., 2005). Other common identified agents are *Streptococcus* spp., *Brucella* spp. and *Escherichia coli* (Kornegay, 1986; Gilmore, 1987; Burkert et al., 2005). Most dogs respond well to a long course of antibiotics, anti-inflammatory therapy and rest. In one study, the mean duration of treatment with antibiotics was 53.7

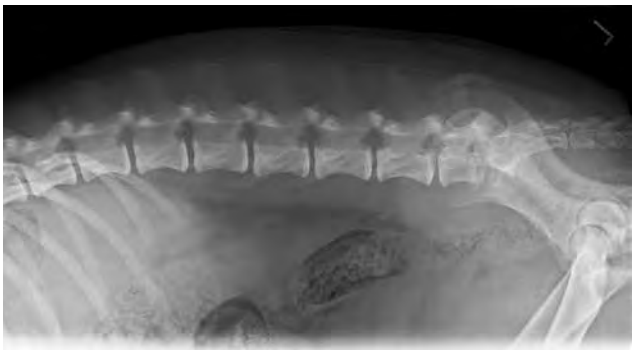

Figure 1. Laterolateral radiograph of the lumbosacral region in a neutral position. There is narrowing of the intervertebral disc space at the lumbosacral joint. The adjacent endplates of L7 and S1 are irregular.

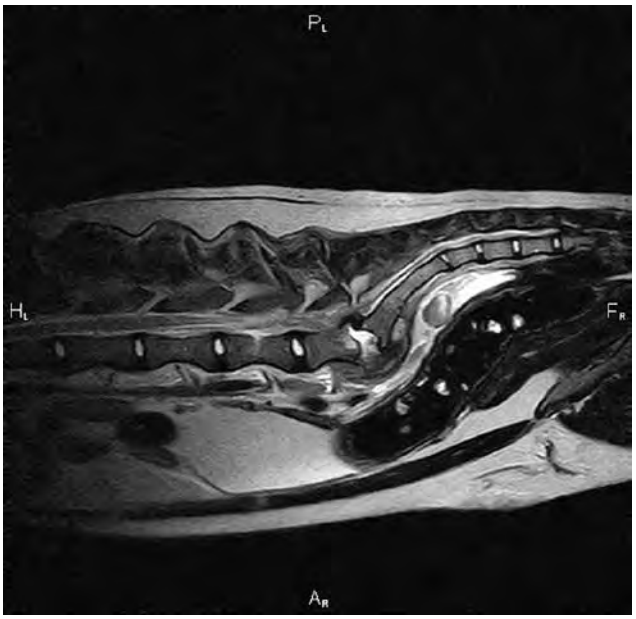

Figure 2. Sagittal T2-weighted magnetic resonance image of the lumbosacral region. Note the widening of the intervertebral disc space at L7-S1, with irregular appearance of the endplates and abnormal hyperintens signal and dorsal herniation of the intervertebral disc.

weeks (Burkert et al., 2005). Surgical treatment is indicated when no improvement is achieved within one or two weeks of medical treatment, when neurological deficits are present or when bone destruction causes vertebral instability (Kornegay, 1993). Possible surgical treatments are decompressive procedures (laminectomy or hemilaminectomy), stabilization of the vertebral column, curettage of the intervertebral disc and bone grafting (Auger et al., 2000; Burkert et al., 2005; Tellegen et al., 2015). In this case report, a dog diagnosed with lumbosacral discospondylitis, not responding to medical management is described. Lumbosacral vertebral instability was diagnosed with the aid of dynamic radiographs and was treated surgically. The clinical and radiographic findings during a one-year follow-up period are discussed.

CASE REPORT

A ten-month-old, female, intact, 40 kg crossbreed dog was referred to the neurology and neurosurgery service with a ten-day history of lethargy, lameness of the right pelvic limb, severe pain and fever. Despite treatment with NSAID's, gabapentin and tramadol during ten days, the dog continued to deteriorate. The dog became non-weight bearing lame on the pelvic limbs and remained extremely painful.

On presentation, physical examination was unremarkable. Rectal temperature was 38.3°C. Neurological examination was limited because of the severity of pain. The dog refused to put any weight on the pelvic limbs but voluntary movement was present in both pelvic limbs. Postural reactions were normal. The withdrawal reflex in the right pelvic limb was reduced. Myotactic and withdrawal reflexes in all other limbs were intact. Spinal palpation revealed severe pain at the lumbosacral level. Neuroanatomical localization was consistent with the L4–S3 spinal cord segments.

Complete blood cell count revealed mild neutrophilia [13.3 × 10^9/L; reference range (RR): 3-11 × 10^9/L] and mild monocytosis [1.6× 10^9/L; RR: 0.0-1.3 × 10^9/L]. Serum biochemical profile revealed mild elevated creatine kinase activity [250 U/L; RR: 0-190 U/L] and alkaline phosphatase [173 U/L; RR: 0-50 U/L].

Radiographs of the entire vertebral column were taken. Laterolateral radiographs of the lumbosacral region revealed narrowing of the intervertebral disc space at the lumbosacral transition. The adjacent endplates of L7 and S1 were irregular (Figure 1). The remain-

Figure 3A. Laterolateral radiograph of the lumbosacral region in a flexed position. There is a ventral subluxation of the sacrum in respect to the L7 vertebra.

Figure 3B. Laterolateral radiograph of the lumbosacral region in an extended position. There is narrowing of the intervertebral disc space at the lumbosacral joint. The adjacent endplates of L7 and S1 are irregular.

ing part of the vertebral column was unremarkable. Magnetic resonance images (MRI) of the lumbosacral vertebral column were obtained with a 0.4 Tesla MRI unit (Hitachi, Aperto). T2-weighted (T2W) images were obtained in sagittal and transverse planes. T1-weighted (T1W) images pre- and post-contrast administration [intravenous administration of gadolinium 27.9 mg/kg body weight (BW)] were obtained in sagittal planes. The intervertebral disc space at L7-S1 was markedly widened with a markedly hyperintense appearance on T2W images of the intervertebral disc (Figure 2). The endplates had an irregular appearance and heterogeneous signal on T1W and T2W images with moderate contrast enhancement. The lumbosacral intervertebral disc was dorsally herniated causing mild focal vertebral canal narrowing. The nerve roots at the lumbosacral intervertebral foramina were bilaterally thickened and showed a heterogeneous hyperintense signal on T2W images and also marked contrast enhancement. Soft tissues ventral, and to a lesser extent, dorsal to the caudal lumbar spine and sacrum showed heterogeneous hyperintense signal on T2W images and contrast enhancement on T1W post-contrast images. Thoracic radiographs, abdominal ultrasound and echocardiography were within normal limits.

Urine and blood samples were collected aseptically for aerobic and anaerobic bacterial cultures. Urine was collected by cystocentesis. The blood was added to a commercially available bacterial culture medium (Signal blood culture system, Oxoid, Basingstoke, Hampshire, UK). Both urine and blood samples were positive for S*taphylococcus* spp. (coagulase positive). The susceptibility test showed sensitivity to amoxycillin clavulanic acid, cephalexin, marbofloxacin, erythromycin, fusidic acid and clindamycin. Intravenous amoxicillin clavulanic acid (Augmentin®, Beecham Group Ltd, Uxbridge, Middlesex, UK), 20 mg/kg BW, q8h and metronidazole (Metronidazole 500 mg/100ml®; Braun, Melsungen, Germany), 10 mg/kg BW, q12h were initiated. Analgesia was provided with a combination of meloxicam (Metacam®, Boehringer Ingelheim, Bracknell, Berkshire, UK), 0.1 mg/kg BW, IV, q24h; methadone (Physoseptone®, Martindale Pharmaceuticals, Romford, Essex, UK), 0.3 mg/kg BW, IV, q4h; gabapentin (gabapentin 300mg®, Double-E Pharma LTD, Dublin, Ireland) 20 mg/kg BW, orally, q8h and paracetamol (Paracetamol 500mg®, M&A Pharmachem LTD, Bolton, England, UK) 10 mg/kg BW, IV, q12h. The dog failed to improve clinically and remained extremely painful ten days after the treatment was initiated. Therefore, it was decided to take dynamic radiographs to look for vertebral instability.

Laterolateral radiographs of the lumbosacral vertebral column were obtained (Figures 3A and 3B). Radiographs were taken in neutral, flexed and extended position. Radiographs taken in flexed position showed a ventral subluxation of the sacrum in respect to the L7 vertebra (Figure 3A). Otherwise, the findings were similar to the radiographs taken ten days earlier. The vertebral instability could explain the persistent pain despite medical management, and surgical management was advised.

Figure 4A. Laterolateral radiograph of the lumbosacral region taken immediately after surgery. This radiograph demonstrates a good position of the screws and pins, which are dorsally embedded in PMMA.

Figure 4B. Ventrodorsal radiograph of the lumbosacral region taken immediately after surgery. This radiograph demonstrates a good position of the screws and pins.

A standard dorsal laminectomy at L7-S1 was per-

Figure 5. Laterolateral radiograph of the lumbosacral region six months after surgery. There is marked osteolysis of the vertebral body of L7. One of the two cranial screws is broken. New bone formation ventral to the lumbosacral junction is more marked compared to the initial radiographs.

Figure 6. Laterolateral radiograph of the lumbosacral region twelve months after surgery. L7 has a more radiopaque appearance compared to the previous radiographs (Figure 5).

formed. Inspection of the spinal canal showed the presence of abnormal appearing epidural fat which was removed. A discectomy was performed, followed by stabilization of the lumbosacral vertebra. A 3.5 mm cortical screw was placed on each side in the pedicles of L7 just caudal to the base of the cranial articular process of L7. Two 3.5 mm cortical screws were placed in each side of the vertebral body of the sacrum just caudal to the caudal articular facet of L7 and behind the dorsal foramen of S1. Transarticular 3.2 mm pins were placed across the L7-S1 articular facets. Subsequently, all screws and transarticular pins were embedded in gentamycin impregnated PMMA bone cement. Postoperative radiographs showed a good position of the implants (Figures 4A and 4B). Samples from the epidural fat and a swab of the intervertebral disc were send for culture and the results came back negative.

Two days after surgery, the dog improved markedly and was able to get up and walk without showing any signs of pain. The dog developed a seroma postoperatively, which improved spontaneously. Analgesia was gradually ceased and the dog was discharged ten days after surgery. Antibiotic treatment was continued in oral form (amoxicillin clavulanic acid (Synulox 500 mg®, Pfizer, Louvain-La-Neuve, Belgium) 20 mg/kg BW, q8h). The metronidazole was stopped as soon as the results of the blood and urine cultures were received.

On follow-up examination six months after the diagnosis, the dog was reported to be clinically normal by the owner and was still being treated with antibiotics. Physical and neurological examination was within normal limits. Radiographs of the lumbosacral region revealed marked osteolysis of the vertebral body of L7 (Figure 5). The most cranial screw on the right hand side was broken, the cranial screw on the left hand side showed a radiolucent halo around its head and body. New bone formation ventral to the lumbosacral junction was more marked compared to the initial radiographs. Blood and urine cultures were repeated and the results came back negative. Based on the radiographic findings, it was decided to continue the antibiotic administration for at least three more months.

The owner reported the dog to be still clinically normal at the follow–up examination twelve months after diagnosis. The dog was still being treated with the antibiotics. Physical and neurological examination was still within normal limits. Radiographs of the lumbosacral vertebral column were repeated, and L7 had a more radiopaque appearance compared to the previous radiographs (Figure 6). The radiolucent halos around the screws became smaller. There was some new bone formation at L6-7, which was thought to represent reactive changes or a sign of ongoing osteitis/osteomyelitis. Blood and urine cultures were repeated and the results came back negative. At that point, it was decided to stop the antibiotics. During the last telephone update, 18 months after diagnosis (i.e. six months after the antibiotics had been stopped), the owner reported that the dog had remained clinically normal.

DISCUSSION

The exact pathogenesis of discospondylitis remains unknown. Infection might be established in the highly vascular, slow flowing metaphyseal and epiphyseal capillary beds with extension into the disc (Kerwin, 2015). It is less likely that the intervertebral disc becomes infected directly as a healthy disc has few (if any) blood vessels (Sharp and Wheeler, 2005).

The source of the infection may be autogenous or iatrogenic. The majority of cases are thought to result from hematogeneous spread of an infection from a distant site, such as the genitourinary tract, skin, heart or teeth (Burkert et al., 2005). In humans, discospon-

dylitis is most frequently diagnosed as a postoperative complication (Rhode et al., 1998; Lehovsky, 1999). Iatrogenic discospondylitis occurs in 2-7% of humans undergoing vertebral column surgery (Rhode et al., 1998; Lehovsky, 1999). In a retrospective study by Canal et al. (2016), discospondylitis was diagnosed in 8 out of 372 dogs (2.2%) as a postoperative complication after spinal decompression surgery for intervertebral disc herniation. In dogs, urinary tract infection is the most commonly diagnosed concurrent disease condition (Burkert et al., 2005). Also in the case reported here, a urinary tract infection was the most likely underlying cause. *Staphylococcus* spp. was identified in the blood and urine cultures of the dog. *Staphylococcus* spp. and *Escherichia coli* are the most common identified agents in dogs with urinary tract and prostatic infections (Krawiec and Heflin, 1992; Johnston et al., 2000).

In this case, initial antimicrobial therapy consisted of amoxicillin-clavulanic acid combined with metronidazole. In general, first-generation cephalosporin and amoxicillin-clavulanic acid are recommended for initial treatment as they have an activity against *Staphylococcus* spp., *Streptococci spp.* and *Escherichia coli*, which are common identified bacteria in dogs with discospondylitis (Thomas, 2000; BSAVA and SAMS, 2011; Sykes and Kapatkin, 2014). Metronidazole was added in this case to broaden the anaerobic spectrum. As results of culture and susceptibility testing should always define further treatment (Thomas, 2000; Burkert et al., 2005; BSAVA and SAMS, 2011; Sykes and Kapatkin, 2014), metronidazole was ceased once the results were known. Combined results of blood and urine microbial cultures yield a reported 30-78% success rate for the detection of an infectious agent (Kerwin et al., 1992; Fischer et al., 1997; Burkert et al., 2005).

The lumbosacral disc is the most commonly affected site in dogs (Burkert et al., 2005). This can be attributed to the high mobility of this intervertebral disc space. A possible explanation is the intermittent venous occlusion or stasis of blood flow at the lumbosacral junction during locomotion, which may lead to focal endplate necrosis. An episode of bacteremia could then lead to focal colonization (Eisenstein and Roberts, 2003).

The diagnosis of discospondylitis is most commonly based on radiographic changes of the vertebrae. Although the radiographic findings in this case report were diagnostic for discospondylitis, MRI was performed to rule out epidural empyema (Plessas et al., 2013). The limitation of radiographs is the time gap between the onset of clinical signs and the first appearance of the radiographic findings, as well as the disassociation between the clinical and radiographic signs during recovery (Thomas, 2000; Shamir et al., 2001). In a previous study, it was demonstrated that dogs that were admitted less than 20 days from the onset of clinical signs, either had no radiographic abnormalities, or had initial signs of collapsed disc space with or without bony lesions (Shamir et al., 2001). In the same study, worsening of the radiographic changes continued despite improvement of the clinical signs after the antibiotic treatment was started (Shamir et al., 2001).

In young dogs (less than one year old), radiographic evidence of improvement (bridging and sclerosis) has been evident at a three-weeks follow-up examination. In older dogs, radiographic evidence of improvement has been documented at six- to twelve-weeks follow-up examination (Shamir et al., 2001). In the dog of this case report, severe osteolysis of L7 was present six months after diagnosis. Osteolysis together with the radiolucent halos seen around the screws, raised the suspicion of ongoing infection. Possible ongoing infection may result from an undetected foreign body, infection by fungal organisms, colonization by antimicrobial-resistant bacteria, or the inability of antimicrobial drugs to penetrate into the target tissue (Burkert et al., 2005). Another possible explanation is bone loss because of disuse of L7 immobilized by screws and cement. The only sign of improvement was the increased amount of new bone formation at L7-S1 compared to the initial radiographs. Despite the severe changes seen on the radiographs, the dog did not show any signs of ongoing infection. A follow-up MRI examination could have provided more valuable information about a possible ongoing infection but the presence of metallic implants would have caused significant susceptibility artefacts likely precluding an adequate evaluation of the area of interest.

Dynamic radiographs were important in this case to detect the vertebral instability. Only the radiographs in flexion revealed lumbosacral instability. It is questionable if the dynamic radiographs should have been taken on the first day. The instability might have been detected earlier but conservative management would still have remained the first choice of treatment as surgery is a very invasive procedure with high risk of complications due to the already present infection. Instability is most likely caused by the extensive bone destruction due to infection, and is a feature of late-stage discospondylitis. The combination of vertebral instability together with the compression of the cauda equina by the pyogranulomatous material coming from the intervertebral disc probably played a major role in the persistent pain experienced by the dog in this case report. The goal of the surgery was to decompress the cauda equina and to stabilize the lumbosacral vertebrae. Retrospectively, the significant improvement of the dog shortly after surgery demonstrated the need for surgical treatment of this dog.

In conclusion, this is a case report about a dog with severe discospondylitis and secondary vertebral instability treated surgically. In dogs with discospondylitis not responding to conservative treatment, persistent pain might be caused by vertebral instability. Surgical stabilization of the affected instable vertebrae may result in fast and drastic clinical improvement.

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Diabetes mellitus and hypercortisolism in a cat

Diabetes mellitus en hypercortisolisme bij een kat

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ABSTRACT

A fourteen-year-old Persian cat was referred because of poorly controlled diabetes mellitus despite insulin and dietary treatment. Clinical signs were severe polydipsia/polyuria (pupd), poor hair coat quality, stomatitis and hind limb weakness. At the time of initial presentation, he was treated with glargine insulin (0,75 IU/kg BID). A low dose dexamethasone suppression test (LDDST) revealed hypercortisolism (HC). The cat was additionally treated with trilostane, and remission of diabetes mellitus was obtained one year later.

This case illustrates the importance of diagnosing an underlying cause of poorly controlled diabetes mellitus. Although hypercortisolism is rare in cats, it is important to consider the disease in these cases. The hypercortisolism in this cat was efficiently managed with trilostane, resulting in a good quality of life.

SAMENVATTING

Een veertien jaar oude Perzische kat werd doorverwezen vanwege slecht gereguleerde diabetes mellitus ondanks insulinebehandeling en een aangepast dieet. De kat vertoonde uitgesproken polyurie/polydipsie, een slechte vachtkwaliteit, stomatitis en zwakte op de achterhand. Op dat moment werd hij behandeld met glargine insuline (0,75 IE/kg BID). Met behulp van een lage-dosis-dexamethasone-suppressie-test (LDDST) werd hypercortisolisme (HC) gediagnostiseerd. De kat werd bijkomend behandeld met trilostane en één jaar later werd remissie van diabetes mellitus bekomen.

Deze casuïstiek illustreert het belang van de diagnose van een onderliggende oorzaak van slecht gereguleerde diabetes mellitus. Ook al is hypercortisolisme zeldzaam bij katten, het is belangrijk de ziekte bij deze gevallen in de differentiaaldiagnose op te nemen. Hypercortisolisme werd bij deze patiënt behandeld met trilostane, resulterend in een goede levenskwaliteit.

INTRODUCTION

Diabetes mellitus (DM) is a common endocrine disease in cats. It is defined as a relative or absolute insulin deficiency that causes persistent hyperglycemia. The classification of DM is based on the mechanism of insulin deficiency. In analogy with human classification, it can be divided in type 1, type 2 and 'other specific types' of diabetes. Approximately 80 to 95 % of diabetic cats are thought to have type 2 diabetes mellitus (Rand, 2013; Gostelow et al., 2014).

Type 2 initiates with a relative deficiency of insulin that later becomes an absolute deficiency. Factors that contribute to insulin resistance are genotype, obesity, physical inactivity and diet. The β-cells try to compensate by secreting more insulin. The chronic high demand for insulin leads to β-cell failure and loss through apoptosis causing insulin deficiency (Niessen et al., 2013; Rand, 2013).

The management of diabetic cats can be challenging. In these cases, the etiopathogenesis of DM may not be type 2 but an underlying disease. This type is categorized as 'other specific types of diabetes'. Some of these diseases, such as pancreatitis and pancreatic

Figure 1. Poor hair coat quality and discoloration.

Figure 2. Alopecia of the ventral abdomen.

neoplasia, destroy β-cells. Other diseases, such as hypersomatotropism and hypercortisolism, induce diabetes by producing an excess of growth hormone and cortisol, respectively. These hormones cause insulin resistance (Rand, 2013; Niessen et al., 2013). Currently, hypersomatotropism is thought to be the underlying disease in feline DM in 25 to 30% of cases (Rand and Gottlieb, 2017).

Insulin resistance can be an important component of the pathogenesis of diabetes mellitus secondary to an underlying disease. Resolution of peripheral insulin resistance together with good glycemic control may result in remission of feline diabetes (Scott-Moncrieff, 2010).

Contrary to dogs, hypercortisolism (HC) is rare in cats. The main differences and similarities in HC between dogs and cats are summarized in Table 1. In most cases (85 %), HC is caused by a tumor of the pituitary gland (pituitary-dependent HC). The elevated secretion of adrenocorticotropic hormone (ACTH) causes hyperplasia of both adrenal glands and an increase in production of cortisol. In approximately 15 % of HC in cats, it is caused by functional adrenal tumors that autonomously secrete cortisol. Fifty percent are malignant adenocarcinomas (Bhatti and Daminet, 2004; Chiaramonte and Greco, 2007; Feldman, 2014).

In this article, a cat with DM and HC is described. Medical treatment of HC in cats with trilostane is further addressed.

CASE REPORT

A fourteen-year-old, male, castrated Persian cat of 5.25 kg was presented at the Small Animal Clinic, Faculty of Veterinary Medicine (Ghent University) for poorly controlled DM (day 0). Four months before, diabetes mellitus had been diagnosed and therapy with insulin (Caninsulin®, MSD Animal Health, Brussels, Belgium) was initiated. The insulin dose was gradually increased to 6 IU BID (1.14 IU/kg) based on blood glucose curves, performed at home (HMBG), still showing poorly controlled DM. Baseline data (complete blood count, biochemistry profile, electrolytes) were obtained by the referring veterinarian at the time of diagnosis. Values were within normal limits. IGF-1, measured by the referring veterinarian after six weeks of insulin treatment, was 21.4 nmol/L (163 µg/L) (1.4 (10.7) – 53.8 (411)). Three months after diagnosis, therapy was changed to glargine (Lantus®, Sanofi-Aventis, Frankfurt am Main, Germany). At the time of presentation at the Small Animal Clinic, the cat received 4 IU BID (0.75 IU/kg), without significant improvement.

The patient presented with persistent pupd, poor hair coat and discoloration and hind limbs weakness (Figures 1 and 2). The body condition score was 6/9 and the cat was fed a diabetic diet ad libitum (Royal Canin Diabetic®).

On physical examination, stomatitis, halitosis and mild hepatomegaly were noticed. To search for underlying diseases and assess the adrenal glands, an abdominal ultrasound was performed and revealed mild hepatomegaly with a homogenous hyperechoic parenchyma, suggestive of lipidosis. Both adrenal glands had a normal shape, the left gland was normal in size (4.1 mm), whereas the right gland was mildly enlarged (5.6 mm) (Figure 3). The mildly enlarged right adrenal gland could be an incidental finding, i.e. anatomical variation, measurement variability. However hyperplasia secondary to pituitary disease (PDH or acromegaly) or an early adrenal tumor could not be excluded. Urinalysis including culture showed glucosuria and isosthenuria but no bacterial cystitis. As

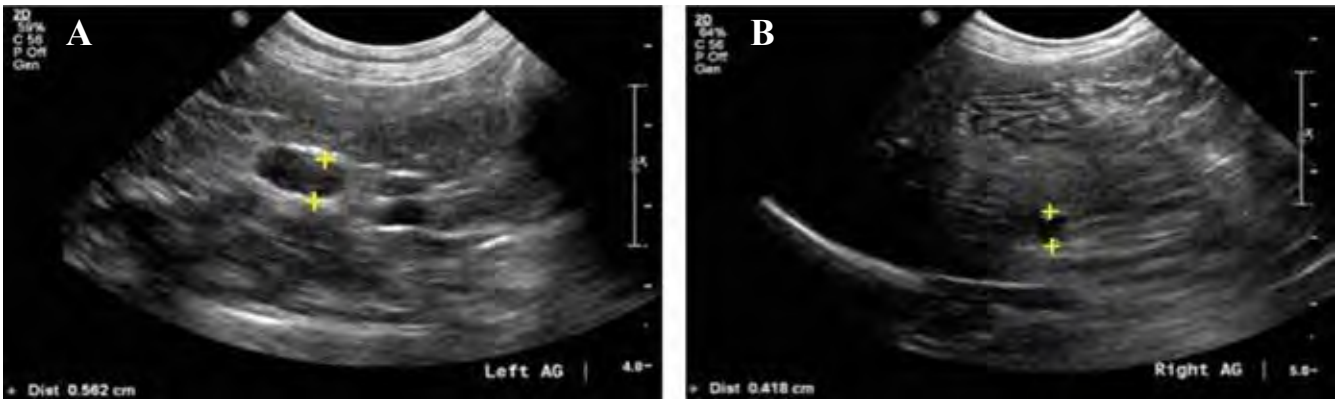

Figure 3. Ultrasound image of the adrenal glands. A. Longitudinal image of the left adrenal gland, and B. transverse image at the level of the caudal pole of the right adrenal gland. The calipers (yellow ++) indicate the height of the caudal pole.

the dose of insulin administered was not yet at a level suggestive of insulin resistance (>1.5 IU/kg BID), the initial advice was to increase the dose of glargine to 4.5 IU BID (0.8 IU/kg), to treat the stomatitis (detartration and extraction of affected teeth) and to promote weight loss (Sparkes et al., 2015). Insulin management errors were considered and ruled out. Other causes of insulin resistance and additional work-up were discussed with the owner in case no improvement would be observed.

Four weeks later (day 27), the cat was receiving 5 IU BID (0.95 IU/kg) of glargine but the same signs were still present. A low dose (0.1 mg/kg) dexamethasone suppression test (LDDST) was performed and confirmed HC (Table 2). In some cases, this test can also allow to make a differentiation between pituitary-dependent (PDH) and adrenal-dependent HC (ADH) based on the level of suppression of cortisol. In this case, there was suppression after four hours (<40 nmol/L) and an escape of suppression after eight

Table 1. The main differences of hypercortisolism between dogs and cats (Bhatti and Daminet, 2004; Nelson, 2014; Boland and Barrs, 2017).

	Dogs		Cats
Signalment	Breed predisposition	≠	No breed predisposition
	Older dogs	=	Older cats (>10 years)
	More in female dogs	≠	More male cats (54%)
Location	PDH (85%)	=	PDH (85%)
	ADH (15%)		ADH (15%)
History	Some have DM (10%)	≠	Most have DM (80%)
Clinical signs and physical examination (common findings)	Pupd	=	Pupd
	Polyphagia		Polyphagia
	Abdominal distention		Abdominal distention
	Endocrine alopecia		Endocrine alopecia
	Weakness		Lethargy
	Hepatomegaly		Hepatomegaly
	Epidermal atrophy		1/3 have extreme skin fragility
	Panting	≠	Weight loss
ALF	Often iso-enzym is induced	≠	No increase of iso-enzym
USG	Markedly decreased	≠	Usually > 1.020
Endocrine tests	LDDST (0,01 mg/kg IV)	≠	LDDST (0,1 mg/kg IV)
	UCCR	=	UCCR
Medical therapy	Trilostane	=	Trilostane

Table 2. Results of low dose dexamethasone suppression test (LDDST) in a fourteen-year-old cat with diabetes and suspicion of hypercortisolism. A value > 40 nmol/L, 8 hours after dexamethasone administration, is consistent with HC (Boland and Barrs, 2017).

Sample 1 (T0)	Sample 2 (4h)	Sample 3 (8h)
69 nmol/l	39 nmol/l	55 nmol/l

Table 3. Results of ACTH stimulation tests, used as follow-up in a fourteen-year-old cat with hypercortisolism treated with trilostane. Day 0 = first presentation in clinic.

Day of test	Dose trilostane	Dose trilostane after test	Sample 1: basal cortisol	Sample 2: post-ACTH
54	10 mg BID	10 mg BID	22 nmol/l	105 nmol/l
138	10 mg BID	8 mg BID	11 nmol/l	41 nmol/l
257	8 mg BID	7 mg BID	17 nmol/l	30 nmol/l
383	7 mg BID	5 mg BID	8 nmol/l	19 nmol/l
453	5 mg BID	5 mg BID	25 nmol/l	52 nmol/l
474	5 mg BID	0 mg	30 nmol/l	50 nmol/l

hours of administering dexamethasone. This is suggestive of PDH (Feldman, 2014; Boland and Barrs, 2017). This finding was compatible with the clinical signs and the poorly controlled DM. Diagnostic imaging was advised but declined by the owners. Both surgical and medical options were discussed. The owners elected a medical treatment with trilostane (Vetoryl®, Dechra Limited, North Yorkshire, UK; 10 mg BID). The dose of glargine was lowered to 4 IU BID because the treatment of HC could lead to an improved sensitivity to insulin.

A control examination four weeks later (day 54) revealed some improvement of the pupd. The results of HMBG showed constant hyperglycemia with a nadir of 18.6 mmol/L. Serum biochemistry revealed mild azotemia (creatinine 153 µmol/L, IRIS stage II). This has been previously described in cats on trilostane therapy (Mellett Keith et al., 2013). Thoracic radiographs were performed for further work-up of poorly controlled diabetes mellitus and for detection of potential concurrent abnormalities. No clinically significant abnormalities were found. An ACTH-stimulation test showed an optimal post-ACTH cortisol level (Table 3). It is recommended to perform this test four to six hours after administration of trilostane (Neiger et al., 2004). The aim of treatment should be clinical improvement in combination with post-ACTH serum cortisol concentrations between 50 and 150 nmol/L (Niessen et al., 2013). Electrolytes in the present case were within normal limits. The same dose of trilostane was continued. The dosage of glargine was increased to 5 IU BID.

At the next control examination, six weeks later (day 103), pupd was still present. HMBG still showed an insufficient glycemic control, hence the dose of glargine was increased (6 IU BID). Mild azotemia persisted. Urinalysis was advised but was difficult to perform because of the cat's aggressive nature.

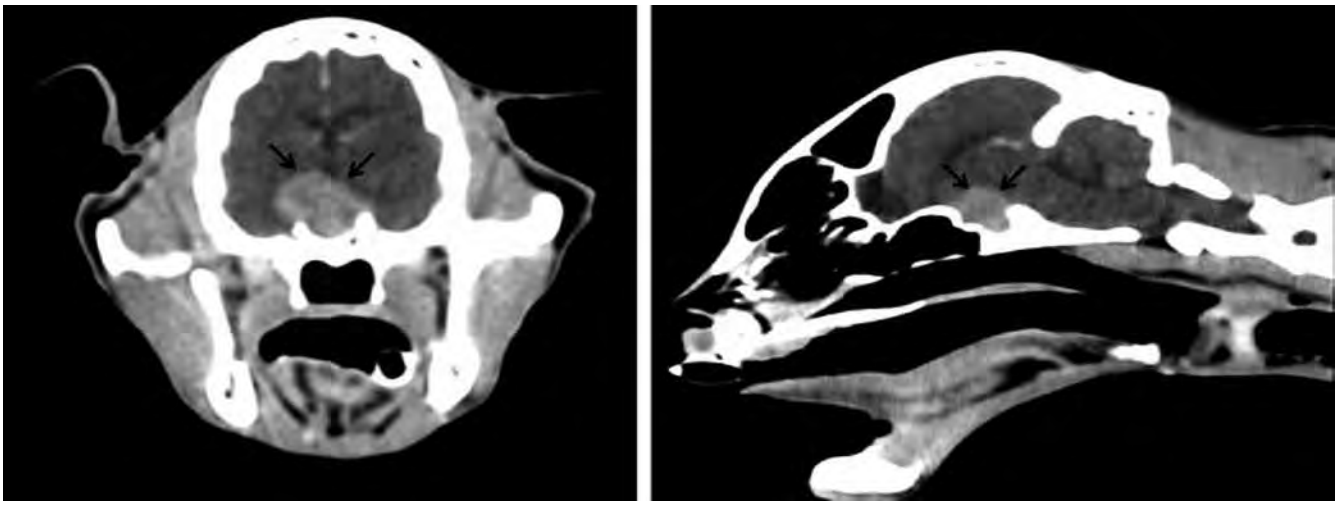

Figure 4. Post-contrast computed tomographic images (transverse image on the left side and mid sagittal image on the right side) showing a strongly, mildly heterogeneously, enhancing mass lesion in the pituitary fossa corresponding to a pituitary macroadenoma (black arrows).

Table 4. Sensitivity and specificity of tests that can be performed on cats to detect and/or differentiate hypercortisolism.

Test	Reference	N	Sensitivity	Specificity	Remarks
LDDST	Niessen et al. (2013)	/	Very good	Poor	
	Chiaramonte and Greco (2007)	/	/	/	Test of choice by many authors
	Feldman (2014)	58	100 %	Was not critically assessed	47 cats of their series and 11 cats from the literature: Immink et al. (1992) (two cats) – Goossens et al. (1995) (three cats) – Meij et al. (2001) (three cats) – Neiger et al. (2004) (three cats)
	Valentin et al. (2014)	28	/	/	Twenty-seven of twenty-eight tests were consistent with HAC and one was equivocal
ACTH stimulation test	Niessen et al. (2013)	/	Lack	/	In two thirds, the cortisol is in normal range
	Chiaramonte and Greco (2007)	/	/	/	Only 50-60 % with HAC show exaggerated response to ACTH administration
	Feldman (2014)	65	33 %	Questionable	51 cats of their series and 14 cats from the literature: Immink et al. (1992) (one cat) – Schwedes (1997) (one cat) – Watson and Herrtage (1998) (five cats) – Moore et al. (2000) (one cat) – Skelly et al. (2003) (one cat) – Neiger et al. (2004) (five cats)
	Valentin et al. (2014)	37	All samples : 56 % At 60 min 89 %	At 30 min : 89 % At 60 min: 89 %	
UC/CR	Niessen et al. (2013)	/	Most sensitive screening test	/	Elevated UC/CR could be result of concurrent illness and stress
	Chiaramonte and Greco (2007)	/	/	/	Also high in cats with non-adrenal illness and so, is false-positive high. High cortisol:creatinine ratio in cats with concurrent disease should be confirmed with a LDDST (0,1 mg/kg
	Feldman (2014)	48	Ok	Not ok	They included 28 cats of their series and 20 cats from the literature: Goossens et al. (1995) (six cats) - Schwedes (1997) (one cat) - Skelly et al. (2003) (one cat) - Meij et al. (2001) (seven cats) - Neiger et al. (2004) (five cats)
	Goossens et al. (1995)	6	Sensitive	Not mentioned	

On day 138, the ACTH-stimulation test revealed a low post-ACTH cortisol level (Table 3). The dose of trilostane was decreased to 8 mg BID (pharmaceutically compounded). The HMBG curve showed periods of hypoglycemia and the dose of insulin was decreased to 4 IU BID. The azotemia (202.4 µmol/L, IRIS stage II) was mildly increased compared to the previous control, and ideally, a urinalysis should have been performed, but was again impossible because of the cat's aggressive nature. It was advised to make a mix of diabetic and renal food (50/50) because of the high protein levels in the diabetic food.

The next control examination on day 257 (Table 3) again led to a decrease of the trilostane (7 mg BID) and glargine (1.5 IU BID) dose. Urinalysis showed renal proteinuria (UPC 0.52, inactive sediment, negative culture) and isosthenuria (1,014). The mild azotemia was still present. However, according to the owners, pupd was significantly decreased.

During the next three months, the insulin therapy was tapered and could finally be stopped. More than one year after the first consultation, remission of dia-

betes mellitus was suspected. Weekly monitoring of the glucose level, four hours after each meal, was recommended to detect possible relapse of diabetes mellitus. A month after stopping the insulin therapy (day 383), a control ACTH-stimulation test showed again a low post-ACTH cortisol level (< 50 nmol/L) and the dose of trilostane was decreased to 5 mg BID (Table 3). The azotemia (creatinine 222 µmol/L, IRIS stage II) was stable to mildly progressive and a control urinalysis showed borderline proteinuria. The owners mentioned pupd and weight loss. Because the cat was in diabetic remission and the azotemia was mildly progressive, the diet was changed to a strictly renal diet. Stopping diabetic diet can increase the risk of relapse; however, a renal diet was elected because the progressive azotemia was estimated to be more important (Sparkes et al., 2015). Thyroxine (T4) concentration, to exclude concomitant hyperthyroidism because of the weight loss, was within the reference range.

On day 453, the cat presented with compulsive behavior; walking around the table aimlessly. A plantigrade stance was present. Eating and drinking behavior was normal, but weight loss was noticed (400 g). The cat received trilostane 5 mg BID and the ACTH-stimulation test showed a good control of HC (Table 3). The owners declined further diagnostic imaging.

During the next month, the neurological signs worsened and the cat developed an unsteady gait. On day 474, the post-ACTH cortisol level was borderline and trilostane was stopped because of deterioration of the general condition of the cat (Table 3). The neurological signs disappeared but one month later, the cat started to circle to the right. He also seemed blind. The temporarily improvement of the clinical signs was probably because stopping trilostane caused an increase in serum cortisol and hence the symptoms were reduced. Since the history of HC and the current neurological signs, there was a suspicion of a macroadenoma and a CT-scan was re-advised. The CT scan (day 562) confirmed a large asymmetric, strongly contrast-enhancing mass in the pituitary fossa extending dorsally out of the sella turcica (Figure 4). Treatment options were discussed with the owner. Considering the life-quality and life expectancy, the owners elected euthanasia.

DISCUSSION

Insulin resistance is defined as present in a cat with poorly controlled DM on an insulin dosage greater than 1.5 IU/kg per injection (Scott-Moncrieff, 2010; Caney, 2013). It is important to differentiate insulin resistance from other causes of poor glycemic control, such as administration difficulties and insulin-related factors. A thorough history and demonstration of an injection by the owner may help to exclude many interfering factors. If no management problem can be found, further investigations are required (Scott-Moncrieff, 2010; Sparkes et al., 2015).

Assessment for concurrent diseases includes urinalysis and culture, hematology and serum biochemistry, abdominal imaging and thoracic radiographs. Serum feline pancreatic lipase measurement can be valuable in the diagnosis of pancreatitis. Especially hypersomatotropism (acromegaly) but also HC are important causes of severe insulin resistance. Hypersomatotropism is evaluated by a measurement of serum insulin-like growth factor-1. In this case, hypersomatotropism was considered highly unlikely due to IGF-1 measurement, performed several months after the insulin treatment had been started (Caney, 2013; Roomp and Rand, 2013; Sparkes et al., 2015; Ramsey and Herrtage, 2017). Since the hepatic growth hormone receptors are stimulated by insulin for the production of IGF-1, the concentrations of IGF-1 may be low in untreated diabetic cats and may increase during treatment with insulin. Therefore, it is important to treat diabetic cats first, before measurement of IGF-1 (Reusch et al., 2006; Ramsey and Herrtage, 2017).

There are multiple tests to diagnose HC in cats. All have advantages and disadvantages. In Table 4, their sensitivity and specificity are listed up. The LDDST is based on suppression of the cortisol level by administration of dexamethasone in normal cats. Cats need a higher dose of dexamethasone (0.1 mg/kg intravenously) than dogs because a high percentage of normal cats do not experience the suppressive effects of dexamethasone in lower dose (Chiaramonte and Greco, 2007; Scott-Moncrieff, 2010; Niessen et al., 2013). An ACTH stimulation test can also be used for screening. This test is based on the adrenal glands producing excessive amounts of cortisol after stimulation in cats with HC compared to normal cats (Niessen et al., 2013). Urine cortisol/creatinine ratio (UCCR) can be used as an initial screening test. Additionally, the ratio can be used to differentiate between pituitary-dependent (PDH) and adrenal-dependent HC (ADH), if the suppression test with dexamethasone is performed. The advantage of this test is that it can be performed at home by collecting morning urine. If there is more than 50 % of suppression of the average UCCR after administration of dexamethasone, it is suggestive for PDH (Goossens et al., 1995; Niessen et al., 2013). The reference range for UCCR and cortisol post-ACTH is laboratory specific. There are other tests like measuring endogenous adrenocorticotropic hormone (ACTH) and pro-opiomelanocortin, but these will not be further discussed (Niessen et al., 2013). In this case, a LDDST was suggestive of PDH.

Besides endocrine testing, medical imaging is advised. Abdominal ultrasonography, CT and MRI can be used to image the adrenal glands and the pituitary gland. Reports on ultrasound of the adrenal glands of cats with PDH are limited. Similar to dogs, the majority of cats with PDH show bilateral enlargement of the adrenal glands with retention of the normal shape. However, few cases with normal sized adrenal glands or mild asymmetric enlargement, as described in this

case report, have been reported. (Combes et al., 2013; Valentin et al. 2014; Boland and Barrs, 2017). In cats with ADH, the most important finding is a unilateral adrenal mass, with normal or small contralateral gland (Valentin et al., 2014; d'Anjou and Penninck, 2015; Boland and Barrs, 2017). The pituitary gland can be visualized by CT and MRI. About 50 % of pituitary tumors are large enough in size to visualize (Chiaramonte and Greco, 2007; Niessen et al., 2013; Boland and Barrs, 2017). Generally, tumors smaller than 10 mm in height are considered microtumors, whereas the larger masses are macrotumors. Microtumors are challenging to diagnose on imaging, whereas macrotumors are well-defined, strongly contrast-enhancing masses in the sellar region (Wisner and Zwingerberger, 2015).

The cat described in this case report was treated with lente insulin after initial diagnosis of DM and then switched to a longer-acting insulin (glargine). At the time the cat was examined, only one insulin with a veterinary license for cats was available in Belgium, i.e. Caninsulin® (MSD Animal Health, Brussels, Belgium). It is a porcine insulin zinc suspension with an insulin concentration of 40 IU/ml. Because of the poor control of DM, and because glargine and protamine zinc insulin (PZI) have a longer duration of action, the treatment was changed to glargine by the referring veterinarian. At time of diagnosis and follow-up, PZI was not registered in Belgium. Glargine is a long-acting synthetic insulin analogue. PZI has a similar duration of action as glargine in healthy cats (Marshall et al., 2008). A difference between PZI and glargine is the time to reach the first nadir glucose concentration. This is significantly shorter for PZI than for glargine but the time to reach the last nadir is similar, i.e. 14 hours (Marshall et al., 2008). Currently, proZinc® (Boehringer Ingelheim, Ingelheim, Germany) is available in Belgium. In several studies, a better glycemic control and higher remission rates with glargine and PZI have been suggested than with lente insulin (Boari et al., 2008; Marshall et al., 2009; Nelson et al., 2009; Roomp and Rand, 2009). However, it should be emphasized that the current level of evidence of all studies is moderate to poor. Common reasons of bias are lack of randomization and blinding, poor study design and small sample size (Gostelow et al., 2014).

In the literature, a dose of insulin > 1.5 IU/kg per injection is considered a cut-off value for insulin resistance, although it is often recommended to investigate causes of poorly controlled DM at lower dosages of insulin, like in this case (Sparkes et al., 2015). In the present case, HC was diagnosed and medical treatment was started with trilostane. Trilostane is an inhibitor of the 3β-hydroxysteroid dehydrogenase enzyme. This enzyme has an essential role in the synthesis of steroids (Ramsey and Herrtage, 2017). There are currently no pharmacokinetic data available for trilostane in cats and only few reports of cats with HC treated with trilostane. In two case reports, each on one cat (i. e. one cat with PDH and one cat with bilateral adrenal enlargement with excessive sex hormone production), improvement of clinical signs has been described. In both cases, the initial dosage was 30 mg once daily (Skelly et al., 2003; Boag et al., 2004). In the cat with PDH, the dosage was increased to 30 mg twice daily. Therapy was stopped when the cat became anorexic. An ACTH stimulation test was not performed, so iatrogenic hypocortisolism could not be ruled out. The cat died of renal failure. It was not clear if renal toxicity due to trilostane was involved (Skelly et al., 2003). A study on five cats with PDH described two cats that died after 16 and 140 days but three others were still alive after 6, 11 and 20 months, respectively. Three cats were diagnosed with DM but continued to require insulin after treatment with trilostane was started. In all cats, a few days after starting trilostane therapy, clinical signs were improved. Final dosages of 5.4 mg/kg once daily and 7 mg/kg twice daily were used (Neiger et al., 2004). The largest and most recent study by Mellett Keith et al. (2013) included fifteen cats with spontaneous HC; fourteen cats were diagnosed with PDH and one cat with ADH. An improvement of the clinical signs and of the ACTH stimulation test results was described in 13 of the 15 cats. In that study, diabetes mellitus was reported in 9/15 cases. In 6/9 cats with diabetes the insulin requirements could be decreased by 36 % within two months. The median survival time was 617 days for all cats. The mean final dose of trilostane used, was 2.7 mg/kg once daily and 5.6 mg/kg twice daily. Four cats had changes consistent with chronic kidney disease (CKD) on medical imaging (Mellett Keith et al., 2013). Compared to the previous case reports, the cat in the current case was treated with a lower dose of trilostane (+/- 2 mg/kg twice daily starting dose), which could be tapered based on clinical improvement and ACTH stimulation test results. Possible explanations for this difference could be an increased awareness of the disease in cats, and therefore diagnosis at an earlier stage, lower starting dosage of trilostane, as well as improved follow-up by routine performance of ACTH stimulation tests. A similar evolution has been observed in dogs treated for hyperadrenocorticism, as the initial manufacture's starting dose recommendation (3-6 mg/kg once daily) was much higher than the currently advised dose (2 mg/kg once daily) of Vetoryl® (Dechra Limited, North Yorkshire, UK) (Pérez-Alenza and Melian, 2017). In the current case, the cat developed progressive azotemia during treatment. It should be emphasized that before treatment, the urine specific gravity (USG) was 1.015 although the majority of cats with HC have a USG of >1.020 (Feldman, 2014; Boland and Barrs, 2017). It is unclear if the azotemia was caused by therapy or if the CKD had already been developing before treatment.

Up till now, no cases have been reported where remission of DM occurs in cats with HC treated with

trilostane. However, the cat in this case went into remission after 356 days of therapy with trilostane. At day 474, trilostane was stopped because of deterioration of the general condition of the cat. Iatrogenic hypoadrenocorticism, due to necrosis of the adrenal gland cortex, appeared less likely based on ACTH-stimulation test results (Ramsey, 2010).

No studies have been published in cats on the optimal timing to perform an ACTH stimulation test after drug administration. Based on studies in dogs and a similar cortisol nadir after trilostane administration in dogs and cats, a similar timing for the ACTH stimulation test seems justified in cats (four to six hours after trilostane administration) (Neiger et al. 2004). Post-ACTH serum cortisol concentrations should be between 50 and 150 nmol/L. These values are laboratory specific (Niessen et al., 2013). Trilostane is the medical treatment recommended above all other medical options because of its superior efficacy, relative lack of side effects and ease of use. Other medical treatment options are mitotane, ketoconazole, aminoglutethimide and metyrapone but they are not recommended due to the lack of efficacy in some cats, their adverse effects and the difficulty of sourcing them (Niessen et al., 2013; Boland and Barrs, 2017).

Surgical treatment of feline HC depends on the type of HC. If a single adrenal tumor is present, adrenalectomy is recommended. Bilateral removal of the adrenal glands has been described in case of bilateral adrenal tumors or PDH; however, in that case, the patient needs to be treated for hypoadrenocorticism after the removal (Chiaramonte and Greco, 2007; Niessen et al., 2013). Hypophysectomy is the best theoretical option in cats with PDH (Meij et al., 2001; Bhatti and Daminet, 2004; Niessen et al., 2013). In one study, microsurgical transsphenoidal hypophysectomy has been described in seven cats with HC (Meij et al., 2001). Two cats died within four weeks after surgery. The other five cats went into both clinical and biochemical remission of HC two months after hypophysectomy with a median survival time of 15 months with a range of 6 to 46 months; two of the cats were still alive at the time of publication. One cat showed recurring signs of HC 19 months after surgery. The most important postoperative complications were oronasal fistula, transient reduction of tear production and wound dehiscence of the soft palate. Meij et al. (2001) concluded that a learning curve is necessary when treatment of PDH with hypophysectomy is introduced but that it may offer a better quality of life and a higher survival rate than bilateral adrenalectomy or medical management. The owner initially refused advanced medical imaging and surgical treatment. The overall survival time in this case was 535 days since the diagnosis of HC. Based on the described case and previous publications, medical treatment could be a valuable alternative to surgical treatment in cats with HC (Skelly et al., 2003; Boag et al., 2004; Neiger et al., 2004; Mellet Keith et al., 2013).

Although time-consuming and expensive, radiation therapy is another treatment option (Chiaramonte and Greco, 2007). Only a small number of cats treated with pituitary irradiation have been described in the veterinary literature; however, the results indicate it could be a valuable option (Mayer et al., 2006). The median survival time in a study with eight cats was 523 days, another study with five cats had survival rates of 5.5, 8, 15, 18, 20.5 months. Alopecia, color change of hair, hair depigmentation, atrophy of the epidermis and epilation in the treatment field are described complications (Kaser-Hotz et al., 2002; Mayer et al., 2006). In the study by Mayer et al. (2006), in one cat out of eight, an acute aural adverse effect was seen. Bilateral cataracts, most likely an adverse effect of radiation therapy, was diagnosed in one cat of eight, thirteen months after treatment (Mayer et al., 2006). Similarly, as the median survival time with trilostane treatment is approximately 600 days and seems to be overall well-tolerated (cf. the current case and previous publications), medical treatment of HC in cats could be a valuable alternative (Neiger et al., 2004; Mellett Keith et al., 2013).

In the study of Mayer et al. (2006), in only four of the eight cats, follow-up brain imaging was performed: two cats had a decreased tumor size at six and eight months after radiation and two cats had unchanged tumor size at three and five months, respectively. Kaser-Hotz et al. (2002) described follow-up CT examination performed in four cats. In one cat, the mass had disappeared, and in three cats, the mass was stable or had decreased slightly in size. The ideal treatment protocol with radiation still needs to be established (Kaser-Hotz, 2002; Mayer et al., 2006; Chiaramonte and Greco, 2007).

CONCLUSION

In the present case report, a cat with combined diabetes mellitus and HC medically treated with long-acting insulin and trilostane is described. HC is rare in cats but important to consider in case of insulin resistance. The cat in this report was successfully treated with trilostane and had a good quality of life. Diabetic remission was achieved.

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Atypisch junctioneel melanocytoma met pagetoïde spreiding bij een jong paard

Atypical junctional melanocytoma with pagetoid spreading in a young horse

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SAMENVATTING

Huidtumoren, met name melanocytaire tumoren, zijn frequent voorkomende neoplasieën bij het paard. In deze casus wordt een atypisch geval van een melanocytoma bij een bijna vijf jaar oude Andalusiër beschreven. Histopathologisch onderzoek toonde een opvallende epidermale component bestaande uit multifocale tot miliaire nestjes van epitheloïde cellen. Deze waren discontinu verspreid over alle lagen van de epidermis, inclusief de haarfollikelwand. Dit patroon wordt ook pagetoïde spreiding of "buckshot pattern" genoemd en is een kenmerk van verschillende humane tumoren waaronder melanoma's, "Paget disease" en "Bowen's disease". Volgens de auteurs is de onderstaande casus de eerste beschrijving van pagetoïde spreiding van een equine melanocytaire tumor.

ABSTRACT

Melanocytic tumors are commonly encountered neoplasms in horses. In this case report, an atypical case of a melanocytoma in an almost five-year-old Andalusian horse is described. Histopathological examination showed a striking epidermal component consisting of multifocal to miliary nests of epitheloid cells, which were spread discontinuously in all layers of the epidermis, including the outer root sheath of the hair follicles. This phenomenon is called pagetoid spreading or buckshot pattern and is a feature of multiple human neoplasms, among which are melanomas, Paget disease and Bowen's disease. To the authors' knowledge, this is the first case report to describe pagetoid spreading in an equine melanocytic tumor.

INLEIDING

Huidtumoren zijn de meest frequent voorkomende tumoren bij paarden, waarbij melanoma's, na sarcoïd, een prominente tweede plaats innemen (Knottenbelt et al., 2015; Goldschmidt, 2017). Equine melanocytaire tumoren werden klassiek opgedeeld volgens Valentine (1995) in vier categorieën: melanocyten naevus, dermaal melanoma, dermale melanomatose en anaplastisch maligne melanoma. De huidige classificatie streeft naar een speciesoverbruggende naamgeving, waarbij er gesproken wordt over (benigne) melanocytoma's en (maligne) melanoma's (Goldschmidt, 2017). Het dermale melanoma vormt een uitzondering hierop gezien dit eerder een continuüm betreft, waarbij een initieel benigne tumor kan transformeren naar maligniteit (Philips en Lembcke, 2013). Het is tevens veruit de meest gediagnosticeerde categorie en bestaat uit een solitaire, traaggroeiende massa, typisch gezien bij schimmels van middelbare tot oudere leeftijd. In het geval van uitgebreide, multifocale tot samenvloeiende dermale nodules wordt de term dermale melanomatose gebruikt. De voornaamste lokalisaties zijn perineaal, de onderzijde van de staart en externe genitalia, evenals lipcommissuren, het ooglid, parotis speekselklier en de hoofd/hals-regio (Johnson, 1998; Knottenbelt et al., 2015; Goldschmidt, 2017). Histologisch worden ze gekenmerkt door een dense populatie van meestal sterk gepigmenteerde melanocyten die zich (diep) dermaal bevinden als een goed omschreven, niet-omkapselde massa (Goldschmidt, 2017). De prognose is goed indien snel en adequaat ingegrepen wordt door middel van chirurgische excisie. Er wordt namelijk beschreven dat ongeveer één tot twee derden van de melanoma's metastaseert indien ze langdurig aanwezig zijn (Valentine, 1995;

Johnson, 1998; Phillips en Lembcke, 2013). Dissemination naar diverse interne organen wordt regelmatig gedocumenteerd (Phillips en Lembcke, 2013; Knottenbelt et al., 2015).

Het melanocytoma, i.e. de vroegere melanocyten naevus, is een goedaardige variant die meestal gezien wordt bij jonge paarden en is niet gelinkt aan een bepaalde haarkleur (Foley et al., 1991; Valentine, 1995; Knottenbelt et al., 2015). Vaak wordt deze ook op een eerder atypische plaats waargenomen (een andere dan de bovenvermelde locatie voor dermale melanoma's). Het neoplastisch infiltraat is voornamelijk gelokaliseerd in de oppervlakkige dermis of op de dermo-epidermale overgang (Valentine, 1995; Johnson, 1998; Phillips en Lembcke, 2013). Sommige tumoren vertonen eveneens een opvallende epidermale component (Foley et al., 1991; Johnson, 1998) of kunnen histologische kenmerken vertonen, normaliter gelinkt met maligne tumoren, zoals cellulair pleomorfisme, intraepidermale infiltratie of mitotische activiteit (Valentine, 1995).

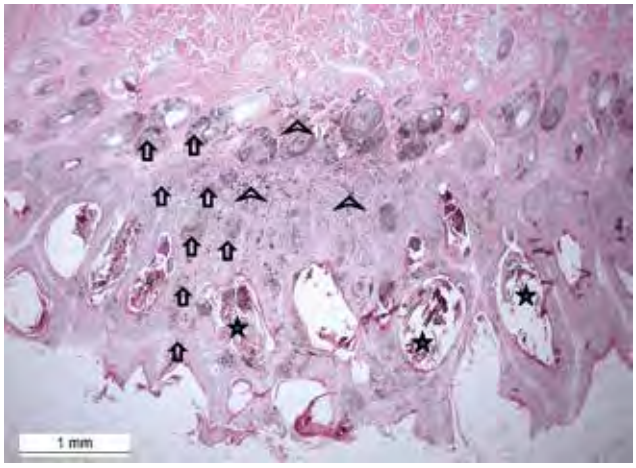
Het anaplastisch maligne melanoma wordt als meest agressieve variant gekenmerkt door een gereserveerde prognose en wordt voornamelijk gediagnosticeerd bij oudere, niet-schimmel paarden (Knottenbelt et al., 2015). Ze bestaan uit sheets van pleomorfe epithelioïde cellen met opvallende nucleaire atypie, variabele (tot verlies van) pigmentatie en een hoge mitotische activiteit (Valentine, 1995; Johnson, 1998; Goldschmidt, 2017).

Voor huidbiopten geldt een specifieke terminologie naargelang de lokalisatie van het letsel. De term junctioneel wordt gebruikt indien het letsel zich ter hoogte van de dermo-epidermale (of dermofolliculaire) overgang bevindt. Compound of samengesteld refereert aan de aanwezigheid van zowel een dermale als epidermale component. Dermaal duidt logischerwijs op een uitsluitend dermale aanwezigheid van (in dit geval neoplastische) cellen (Smith et al., 2002; Goldschmidt, 2017).

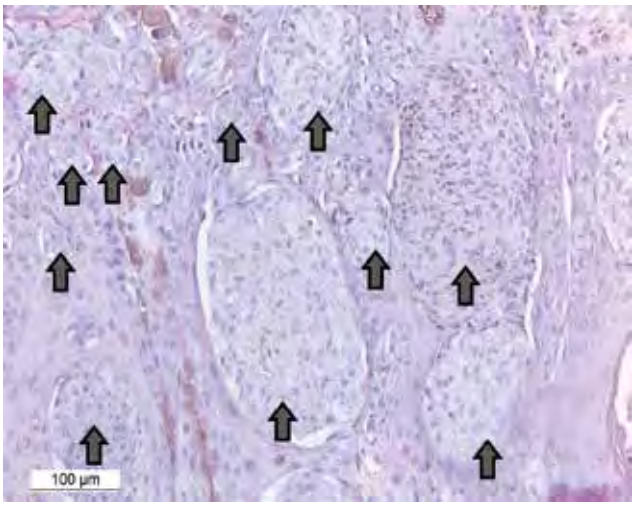
CASUÏSTIEK

Een vier jaar en zeven maanden oude hengst, donkervos, Andalusiër, werd aangeboden voor routinecastratie. Er werd gevraagd om tijdens de anesthesie eveneens een huidknobbeltje te verwijderen dat reeds twee maanden aanwezig was, gelokaliseerd in de huid boven de linkerschouder. Het betrof een traaggroeiende, 3,4 mm verheven, zwart gepigmenteerde nodule met een diameter van 8 mm. De massa vertoonde geen ulceratie of zwelling, noch was er opzetting van de drainerende lymfeknopen voelbaar. Een melanocytaire tumor werd als provisoire diagnose gesteld; qua differentiaaldiagnose kon een focaal hemangiosarcoma of een huidtumor met secundaire bloeding in aanmerking komen. Het gezwelletje werd chirurgisch verwijderd met rondom een 3 mm wijde marge. Het werd onmiddellijk gefixeerd in 4% gebufferde formaldehyde en routinematig verwerkt in het histologielabo van de dienst Pathologie van de Faculteit Diergeneeskunde (UGent). Naast de standaard HE-kleuring werden er eveneens gedepigmenteerde coupes aangemaakt en immunohistochemische kleuringen werden uitgevoerd voor cytokeratine, Ki67 en E-cadherine. Dit laatste is een van de belangrijkste cell-celladhesie-moleculen in epithelia en fungeert op die manier eveneens als tumorsuppressor-gen (van Roy en Berx, 2008). Ook de adhesie tussen normale melanocyten en de omringende epitheelcellen wordt gemedieerd door E-cadherine.

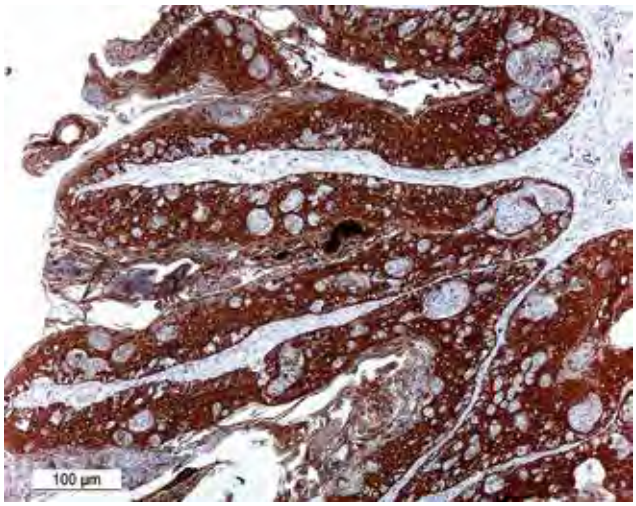
Er werden meerdere dwarse sneden van het ontvangen huidfragment onderzocht met daarin een goed omschreven, niet-omkapselde, dens cellulaire, mild infiltratieve en verheven nodule gelokaliseerd in de epidermis en oppervlakkige dermis (Figuur 1). De massa was opgebouwd uit een monomorfe celpopulatie met een epidermale component van multifocale tot miliaire nestjes van epithelioïde cellen, verspreid doorheen alle lagen van de epidermis en gradueel kleiner wordend naar het stratum corneum (pagetoide spreiding). De grootste nestjes bevonden zich ter hoogte van de basale cellaag. De cellen vertoonden een polygonaal tot spoelvormig uitzicht met een matige tot ruime hoeveelheid bleek eosinofiel cytoplasma met een variabel aantal zwart gepigmenteerde granules (melanine), onduidelijke celranden en een grote, centraal gelegen, rond tot ovale, licht basofiele kern met een fijn chromatinepatroon en 1 (tot 3) nucleoli. De dermale component was veel beperkter en bestond uit eenzelfde celtype met tussenliggend een matige hoeveelheid sterk gepigmenteerde melanofagen. De neoplastische nestjes bevonden zich eveneens in de



Figuur 1. H&E van de hyperplastische epidermis die diepe en plompe "rete ridges" vormt, waarin zich multifocale tot miliaire nestjes van (variabel gepigmenteerde) epitheloïde cellen bevinden (enkele zijn aangeduid met een pijl). Deze intraepidermale eilandjes zijn diffuus verspreid over de volledige epidermis en zijn kenmerkend voor pagetoïde spreiding. Dermaal zijn er aggregaten van sterk gepigmenteerde melanofagen zichtbaar (pijlhoofd). Er is opvallende folliculaire dyskeratose (ster).



Figuur 2. H&E van gedepigmenteerde coupe. Sterkere vergroting van de neoplastische eilandjes (pijl), waardoor de cellulaire kenmerken beter te beoordelen zijn. De tumorcellen hebben een epithelioïde morfologie met een ruime hoeveelheid bleek basofiel cytoplasma en een meestal centraal gelegen, ronde kern conform de beschrijving van pagetcellen.



Figuur 3. Immunohistochemische kleuring voor cytokeratine accentueert de pagetoïde spreiding doordat enkel cellen van epitheliale oorsprong bruin aangekleurd worden; dit in tegenstelling tot de negatief aangekleurde eilandjes van neoplastische melanocyten.

wand van de haarfollikels en induceerden een hyperplastische respons van zowel epidermis als haarfollikels. De epidermis vertoonde diffuse hyperpigmentatie, vormde diepe en plompe dermale uitstulpingen ("rete ridges"). Er was ook hyperkeratose van het folliculair infundibulum (folliculaire keratosis). Op de gedepigmenteerde coupe was de celmorfologie duidelijker zichtbaar (Figuur 2).

Via een immunohistochemische kleuring voor cytokeratine werden de intraepidermale neoplastische cellen zeer opvallend zichtbaar als negatieve eilandjes in het positieve epitheel (Figuur 3). KI67 toonde een laag aantal proliferatieve cellen (2 per 10 "high power fields"). Immunohistochemie voor E-cadherine toonde een duidelijke en vaak zeer intense membranaire aankleuring van de melanocyten aan. Dit laatste was ook het geval voor de hyperplastische keratinocyten in vergelijking met de naastliggende normale epidermis, waar de E-cadherine-aankleuring een normale intensiteit vertoonde.

DISCUSSIE

Vergelijkend met de huidige classificatie van equine melanocytaire tumoren vormt de voorliggende case een atypisch geval. Qua signalement en macroscopisch uitzicht valt deze onder het (goedaardig) melanocytoma. Histologisch zijn er echter kenmerken die eerder zelden waargenomen worden bij equine melanocytaire tumoren, zoals de sterk uitgesproken epidermale component. De massa vertoont eveneens een opvallende pagetoïde spreiding, oftewel "buckshot pattern", een kenmerk dat zowel in de diergeneeskundige literatuur door Goldschmidt (2017) als in de humane literatuur voornamelijk als maligne gekarakteriseerd wordt. Deze term is afgeleid van pagetcellen en staat voor grote epithelioïde cellen met bleek cytoplasma en onregelmatige grote kern met prominente nucleoli. Ze werden als eerste beschreven door Sir James Paget in een maligne neoplasie van de tepel en werden nadien gerapporteerd in andere regio's met apocrien klierweefsel (Nagle et al., 1985). Pagetoïde spreiding is een kenmerk dat frequent gezien wordt in bepaalde humane maligne neoplasieën waaronder melanoma, de ziekte van Bowen en de ziekte van Paget. Het wordt ook regelmatig waargenomen bij humane, benigne melanocytaire neoplasieën, weliswaar minder uitgesproken en eerder centraal in de massa gelokaliseerd in plaats van uitbreidend naar de laterale randen (Petronic-Rosic et al., 2004). Bij equine melanoma's worden er soms wel intraepidermale nestjes van melanocyten beschreven maar werd dit tot nog toe niet vermeld als een buckshot pattern. Dit geval voldoet aan de criteria opgesteld door Haupt en Stern (1995) voor pagetoïde melanocytosis bij de mens. Deze zijn: (a) de aanwezigheid van duidelijk herkenbare melanocyten in de epidermis, (b) de cellen vertonen een discontinu patroon en vormen geen directe verderzetting van de (normale) junctionele populatie ("free floating pagetoid melanocytosis") en (c) de melanocyten moeten zich boven een denkbeeldige lijn parallel aan het huidoppervlak bevinden, namelijk deze boven de basale cellaag van de meest oppervlakkige dermale papillen.

De pathogenese van dit buckshot pattern is onduidelijk maar berust op twee hypothesen voorgesteld door Petronic-Rosic et al. (2004). Enerzijds lijkt het logisch dat de neoplastische cellen een actief infiltratief proces kunnen vertonen, waarbij ze zich door-

heen de epidermis verspreiden en er focale haardjes van proliferatie ontstaan, waardoor er nestjes cellen gevormd worden. Hiervoor dienen de cellen zich ontdaan te hebben van hun normale adhesiemoleculen; in het geval van melanocyten zijn dit voornamelijk E-cadherine en in mindere mate P-cadherine (Tang et al., 1994). Een andere hypothese is een passieve flow via de normale maturatie van keratinocyten die geleidelijk opschuiven naar het (meest oppervlakkige) stratum corneum en in deze beweging ook de neoplastische cellen kunnen meevoeren. Gezien het hier aangetoond sterk positief en duidelijk membranair immunohistochemisch expressiepatroon voor E-cadherine ter hoogte van de neoplastische cellen, lijkt de tweede hypothese bij deze casus het meest aannemelijk.

CONCLUSIE

In deze casus wordt het histologisch voorkomen beschreven van een gekend humaan groeipatroon typisch gelinkt aan maligniteit van een goedaardige tumor bij het paard. Immunohistochemische kleuring voor E-cadherine kon een deel van de pathogenese verklaren door aan te tonen dat de pagetoïde spreiding in dit geval veroorzaakt werd door een passieve opwaartse beweging van de neoplastische cellen tijdens de normale epidermale differentiatie.

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Multifocal osteomyelitis and abdominal abscessation in a warmblood foal

Multifocale osteomyelitis en abdominale abcesvorming bij een warmbloedveulen

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ABSTRACT

To the authors' knowledge, this is the first report, in which a simultaneous occurrence of Salmonella-associated multifocal osteomyelitis and intra-abdominal abscessation in a foal presenting with weight loss, diarrhea and fever is described. No evidence of failure of passive immune transfer had been present at birth. Radiographic examination revealed multifocal type P osteomyelitis. Bacterial culture of synovial fluid revealed a Salmonella spp. Antibiotic treatment was adjusted based on the sensitivity results; however without clinical improvement. The foal developed abscess formations within the left femoral region, and finally a Salter Harris type I fracture of the left femur. Euthanasia was elected based on this finding. Post-mortem computed tomography revealed a large mass within the caudal abdomen that extended into the left pelvic and hind limb region, as well as multifocal osteomyelitis.

Pathological examination identified the mass as a large abscess with multiple fistula tracts. Salmonella spp. was additionally cultured post mortem.

SAMENVATTING

Volgens de auteurs is dit de eerste casuïstiek waarin het simultane voorkomen wordt beschreven van multifocale osteomyelitis en abdominale abcesvorming veroorzaakt door Salmonella bij een veulen met gewichtsverlies, diarree en koorts. Er was geen falen van het passieve immuunsysteem na de geboorte van het veulen. Radiografisch onderzoek toonde multifocale osteomyelitis (type P). In de bacteriële cultuur van het gewrichtsvocht werd *Salmonella*-species geïdentificeerd. De antibioticatherapie werd afgestemd op de resultaten van het antibiogram, echter zonder klinische verbetering. Abcessen ontwikkelden zich verder in de linkerdij en een Salter-Harrisfractuur type I van de linkerfemur werd vastgesteld. Er werd beslist om over te gaan tot euthanasie van het veulen. Postmortaal computertomografisch onderzoek toonde een grote massa in het caudale abdomen, die uitliep tot in de regio van het linkerbekken en achterbeen. Er werden eveneens multifocale osteomyelitis aangetoond.

Middels het pathologisch onderzoek werd de massa als een abces met verschillende fistelgangen geïdentificeerd. Postmortem bacteriologisch onderzoek toonde eveneens *Salmonella*-species aan.

INTRODUCTION

Osteomyelitis with or without associated septic arthritis is a serious orthopedic condition in foals. Septic arthritis is the primary differential diagnosis in any foal presenting with joint effusion and lameness.

Neonatal foals lack adaptive immunity and must obtain all of their antibodies from the ingestion of colostrum. Failure of passive immune transfer is the primary cause of septicemia in foals (Raidal et al., 1996). Septic osteomyelitis is most often the result of hematogenous dissemination of bacteria (Firth, 1983; Hardy 2006). Bacterial infection usually arises from the umbilical region or the respiratory or gastrointestinal tract and may result in septicemia.

In young foals, transphyseal vessels connect and allow communication of the epiphysis and metaphysis blood supply. Closure of the transphyseal vessels

occurs approximately at seven to ten days of age. Hematogenous bacteria can locate in any of these three areas creating septic foci. A classification scheme for septic arthritis/osteomyelitis lesions in the foal has been described by Firth (1983) according to their location within joint and/or bone.

Type S (synovial) is defined as inoculation of the synovial membrane in one or more joints with no radiographic evidence of osteomyelitis, type E (epiphyseal) is defined as osteomyelitis of the subchondral bone, type P (physeal) is defined as osteomyelitis of the physis on the metaphyseal side of the growth plate, and type T is osteomyelitis of the small cuboidal bones in the tarsus or carpus (Firth, 1983).

Depending on an intra- or extra-articular location on the physis, bone inoculation can occur simultaneously with synovial inoculation.

Young foals suffer predominantly from infectious arthritis type S or E, whereas older foals more often suffer from type P infection (Firth, 1983; Hardy, 2006).

Previous studies have documented that the most common bacterial isolates from foals with septic arthritis/osteomyelitis are *Enterobacter* spp., *E. coli*, *Actinobacillus* spp., *Salmonella* spp. and *Streptococcus* spp. (Martens et al., 1980; Brewer et al., 1990; Vatistas et al., 1993).

Radiography is historically the most commonly used imaging modality in diagnosis of septic arthritis and osteomyelitis in horses (Schneider 1999). However, radiographic signs of osteomyelitis are only seen when 50–70% of the bone is demineralized (Wegener, 1991; Goodrich, 2006), resulting in a radiographic lag period of up to 21 days (Firth 1983; Roberts et al., 2010). This can causea delay in diagnosis and prognostic information, as the detected radiographic changes may not accurately demonstrate the severity of the lesion.

Septic osteomyelitis is managed medically with appropriate antibiotics, and surgically when access to the site of infection is possible. The outcome is impacted by delays in treatment, concurrent illness and bacteria pathogenicity (Neil et al., 2010).

Described herein are the clinical and diagnostic imaging findings, as well as treatment and outcome of a case of multifocal osteomyelitis and abdominal abscessation in a foal.

CASE REPORT

A two-month-old warmblood filly was presented with a complaint of fever and weight loss for two days despite a good appetite. At presentation, there was no evidence of failure of passive immune transfer, and a SNAP foal test had been performed at birth (>8g/L). The foal was the third born out of the mare, with no history of complications regarding her past progeny. The foal had been treated by the referring veterinarian with flunixin meglumine, gentamicin and penicillin.

Clinical examination

On presentation, the foal was quiet but responsive. The body condition was below average (91 kg). Rectal temperature was 38.5°C, pulse rate 92 beats per minute, respiratory rate 24 breaths per minute and capillary refill time less than two seconds. The mucous membranes were pale, and the eyes appeared sunken.

Auscultation of the lungs revealed increased lung sounds on the left side compared to the right. Abdominal borborygmi were absent on the right side and decreased on the left. An umbilical hernia was present. Abdominal ultrasound revealed increased fluid content within the colon and cecum, consistent with diarrhea. The left hind metatarsophalangeal joint was distended and an arthrocentesis was subsequently performed. Blood was collected for hematological and biochemical analysis, which revealed an increased serum amyloid A (SAA) of 1040 µg/ml (normal 2.3 µg/ml).

Electrophoresis was not performed, as the total protein was normal. Hyperimmune plasma was not administered as the SNAP foal test at home and total plasma protein were within normal limits.

Synovial fluid from the left metatarsophalangeal joint yielded *Salmonella* spp. Similarly, bacterial blood culture was also positive for Salmonella. Sensitivity results indicated that the organism was susceptible to all antimicrobials against which it was tested (ampicillin-amoxycillin, amoxycillin-clavulanic acid, ceftiofur-cefquinome, fluoroquinolones, tetracyclin, doxycyclin, neo, kana, fram, paromomycin, gentamicin, amikacin, sulfonamide, trimethoprim and trimethoprim+sulfonamides). Treatment with ceftiofur (Excenel; intravenously (IV) (9d) followed by intramuscular (IM) (2d); 7.5 mg/kg BID) was initiated. Additional treatment with ketoprofen (ketofen; 2.2 mg/kg IV SID) was initiated at day 11, and changed to firocoxib (Equioxx; 0.1 mg/kg PO SID) at day 21. Antibiotic treatment was changed at 11d, 22d, 44d and 50d to amoxicillin (Amoxiclav; 20 mg per oral (PO) TID), doxycycline (Doxylin; 10 mg/kg PO BID), clarithromycin (Claritromysine Sandoz; 7.5 mg/kg PO SID) and rifampicin (Rifadin; 5 mg/kg PO SID), respectively. The foal additionally received anti-ulcer therapy (Omeprazole 4 mg/kg po) for two weeks.

The changes in antibiotic treatment were based on a lack of improvement of the clinical signs and persisting fever, and antibiotics were consistently selected according to the antibiogram of the blood culture.

After two weeks at the hospital, the foal had deteriorated and became lethargic. Severe effusion of the right tarsus developed and an arthrocentesis was performed. The centesis revealed a total white blood cell count of 4.7 x 10^8 cells/ dL (normal reference < 300 cells/dL) and a total protein of 38 g/dl (normal reference < 2.5 g/dL), consistent with infection (Schneider 1999). Regional perfusion was performed (Ceftiofur; 5 mg/kg) of the right hind limb, which improved the

Figure 1A. Initial lateromedial radiographic examination of the right tarsus. Soft tissue swelling is present at the cranial aspect of the distal tibia. Ill-defined subcortical radiolucent zones are seen within the distal metaphysis of the tibia (arrowheads), as well as ill-defined, radiolucent areas within the distal physis.

Figure 1B. Radiographic examination on day 36. There is an increased soft tissue swelling at the cranial aspect of the distal tibia. The radiolucent zones within the distal metaphysis are markedly more conspicuous and well-defined, creating a triangular shape (arrowheads). Irregular new bone formation is present at the craniodistal aspect of the tibia, extending into the adjacent soft tissues (arrow).

lameness short term. Three follow-up centeses were performed with progressive improvement of the values. However, after one month, the foal would no longer stand or get up unassisted.

At one and a half month after presentation, an abscess was detected adjacent to the left coxofemoral joint ultrasonographically. Repeated lavage of the abscess cavity was performed.

Diagnostic imaging

Radiography

No significant bony abnormalities were detected on the left metatarsophalangeal joint at the time of presentation. Lateromedial, dorsoplantar, dorsomedial-plantarolateral oblique and dorsolateral-plantaromedial oblique views were obtained of the right hind tarsus when joint effusion was first detected (Figure 1A). The examination showed moderate soft tissue swelling, most prominent at the level of the tibiotarsal joint and distal physis of the tibia. Ill-defined, periosteal new bone formation was present along the distal metaphysis of the tibia. Subcortical osteolysis was seen extending along the distal metaphysis of the tibia as well as diffuse radiolucent areas involving the distal physis. Based on the radiographic findings, a diagnosis of osteomyelitis type P involving the distal physis of the tibia was made. Follow-up radiographic examinations of the right hind tarsus were made at 7d, 14d, 22d, 29d and 36d (Figure 1B).

The evolution of subsequent radiographic examinations included widening and increased demarcation of the subcortical radiolucent zones along the distal metaphysis with the appearance of a triangular zone of decreased opacity within the metaphysis, more pronounced appearance of the lucent areas within the physis, an increasing amount of ill-defined periosteal reaction and persisting soft tissue swelling. On day 22, radiographic examination of the left hind tarsus and both stifle joints was performed based on clinical findings. Radiographic findings of the left hind were similar to the initial examination of the right hind. Examination of the left stifle showed marked soft tissue swelling at the cranial aspect of the joint. Patchy radiolucent areas were detected within the subchondral bone of the lateral and medial trochlear ridges. The distal physis of the femur appeared widened. An ill-defined, triangularly shaped lucent zone surrounded by a sclerotic rim was present within the distal metaphysis of the femur. An irregularly outlined, linear radiolucent area was also detected adjacent to the proximal physis, within the proximal metaphysis of the tibia. No soft tissue swelling of the right stifle was

Figure 1C. Post-mortem computed tomographic image of the same limb. Well-defined, linear hypoattenuating zones are seen adjacent to the cortex of the distal metaphysis and within the distal physis of the tibia (asterisk symbols). The craniodistal tibial cortex is disrupted (arrow). The triangular, hypoattenuating zone within the distal metaphysis was consistent with pus accumulation found on gross pathology.

detected. Severe, ill-defined, radiolucent areas surrounded by sclerosis were present within the femoral trochlear ridges of the right hind. Radiolucent areas were also detected within the metaphysis and physis of the distal femur. A similar linear, radiolucent area adjacent to the proximal physis of the tibia was also seen in the right hind (Figure 2). A diagnosis of arthritis of the femoropatellar joint and osteomyelitis type E and P involving the distal femur and proximal tibia was made.

Radiographic re-evaluations of both stifles were performed at 29d and 36d. Progression of radiographic findings included increased soft tissue swelling (also involving the right stifle), increased conspicuity of the radiolucent areas within the metaphysis of the tibia, ill-defined radiolucent areas within the patella, subchondral bone lucencies within the femoral condyles, increased severity of the irregular outline and radiolucent areas of the femoral trochlear ridges. On subsequent radiographs several small, rounded gas opacities and ill-defined mineral opacities were detected within the caudal soft tissues of the left femoral region. Focal, ill-defined, mineral opacities were also seen at the cranial and caudal aspect of the proximal tibia.

Based on the radiographic findings, abscess formation within the soft tissues of the femoral region was suspected, and an ultrasonographic examination was recommended.

Ultrasound

Multiple, diffuse, heterogeneous and hypoechoic areas containing hyperechoic foci (gas) were detected within the muscles and subcutaneous tissues adjacent to the left coxofemoral joint (abscess). A gas containing fistula tract could be followed extending under the left ilium. Additionally, the abscess extended distally to the level of the tibia. The bony surface of the proximal femur was markedly irregular.

Based on the ultrasound findings and the poor clinical progression of the foal, a radiographic examination of the pelvis was performed. The examination revealed a complete disruption of the proximal physis of the femur with caudal displacement of the distal femur in relation to the femoral head consistent with a Salter Harris type I fracture, suspected to be of pathologic origin (Figure 3).

Outcome

Despite prolonged therapy, the foal deteriorated clinically. Based on the severity and grave prognosis of the femoral fracture, euthanasia was elected.

Post-mortem computed tomography (CT)

A CT examination was performed post mortem for educational purposes. A large, ill-defined structure was detected within the left caudal abdomen, causing a mass effect on the surrounding structures (Figure 4A). The content was heterogeneous and hypoattenuating with a tissue density of approximately twenty

Figure 2. Lateromedial radiograph of the right stifle joint. Patchy radiolucent areas are detected within the lateral and medial trochlear ridges, and within the distal physis of the femur (arrowheads). An irregularly outlined, linear radiolucent area is also detected adjacent to the proximal physis of the tibia (arrows).

Hounsfield units (HU), consistent with cellular fluid. Several gas opacities and multifocal mineralization were present within the content. A fistula tract originated from the caudal aspect of the structure, extending under the axial aspect of the left ileal wing, and then in an abaxial direction to the lateral aspect of the left coxofemoral joint (Figure 4B). Multiple gas opacities were detected within the soft tissues caudal to the left femur; however, a clear connection could not be detected on the CT images. Given the post-mortem examination, contrast-enhanced CT was not possible.

Within the right tibia, well-defined, linear hypoattenuating zones were detected adjacent to the cortex of the distal metaphysis and within the distal physis creating a triangular demarcation of the medulla (Figure 1C). The hypoattenuating zones had a tissue density of approximately 60 HU compared to the 260 HU of the surrounding trabecular bone. The dorsal metaphyseal cortex was disrupted. Similar but less pronounced findings were present in the left tibia.

Several ill-defined, hypoattenuating areas were present within the trochlear ridges of the right femur (stifle joint). Multifocal, hypoattenuating zones were also present within the distal femoral physis. A linear hypoattenuating zone was detected within the proximal metaphysis of the tibia, immediately distal to the physis. A diffuse, patchy increase in attenuation of the epiphysis and metaphysis of the distal femur and proximal tibia was suggestive of mixed sclerosis and osteolysis. Similar abnormalities were seen within the left tibia and femur.

A complete disruption of the proximal physis of the femur was seen in the left limb. The femoral head was still present within the acetabulum; however, the femur was displaced in a caudoproximal direction. Multiple smaller bony fragments were seen at the level of the physis, surrounded by a hypoattenuating rim and with sclerosis of the adjacent bone indicative of sequestrum.

Pathology

A large encapsulated abscess of 25x21x35 was found at the level of the left kidney; however, no involvement of the kidney was seen. Several fistula tracts extended from the abscess. One fistula (1cm diameter) extended to the cranial aspect of the ileum wing, at the level of the tuber coxae, and ended in a round/oval shaped abscess of 12x5cm. An additional fistula tract extended distally between the quadriceps femoris muscle, and ended deeply within these muscles without a clear demarcation. Several abscess formations were seen including one abscess within the popliteal region, caudal to the left stifle joint, with a connection to the caudal aspect of the tibia, one abscess of 13x16 cm located at the medial aspect of the left stifle joint, with fistula tracts extending to the ventral aspect of the pelvis as well as the skin surface, and one abscess of 26x2-3 cm located dorsally to the pelvis with two fistula tracts, one opening caudally to the semitendinosus (previous incision from surgical drainage). As suspected, a pathologic fracture involving the proximal physis of the left femur was found. The right tibiotarsal joint was severely distended with a large amount of viscous yellow fluid. The cartilage was normal. Sharply bordered accumulations of pus were seen within the trabecular bone of the metaphysis of both the left and right femur and tibia. The physis of the femur and tibia were similarly affected. Fifty millilitres of sero-hemorrhagic fluid were present within the abdomen. The iliac lymph nodes were enlarged. Salmonella spp. was additionally cultured post mortem from both the abscess and the sites of bone infection.

Figure 3. Ventrodorsal radiograph of the coxofemoral joints. There is a complete disruption of the proximal physis of the left femur consistent with a Salter Harris type I fracture (arrows). The femoral head is still located within the acetabulum, however there is a caudal displacement of the distal femur in relation to the femoral head.

DISCUSSION

To the authors' knowledge, this is the first report describing a case of polyostotic hematogenous osteomyelitis and concurrent abdominal abscess formation in a foal with *Salmonella* spp. being the causative organism. In foals less than six months old, septic osteomyelitis most often involves the femur, distal phalanx and tibia (Niel et al., 2010). The isolation of *Salmonella* spp. from synovial fluid and multisystemic disease have been associated with an unfavorable prognosis for survival (Steel et al., 1999, Hardy, 2006; Vos et al., 2008). *Salmonella* spp. are Gram-negative bacteria that inhabit the intestinal tract and cause contamination of the environment by excretion of fecal material.

Figure 4A. Computed tomography images of the caudal abdomen. A large, ill-defined structure is seen within the left caudal abdomen (between asterisk symbols). The content is heterogeneous and hypoattenuating, and contains several gas opacities. This finding was consistent with a large intra-abdominal abscess.

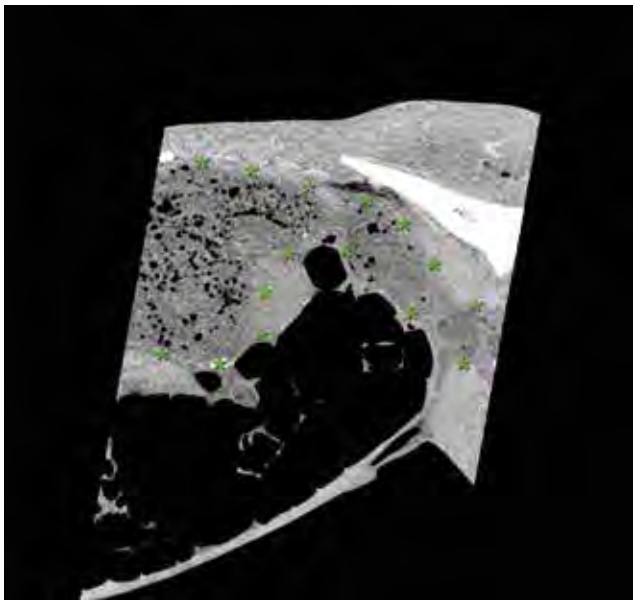

Figure 4B. A fistula tract is seen extending from the caudal aspect of the abscess and underneath the ilial wing (between asterisk symbols). Multifocal, hyperattenuating mineralizations are also seen within the content.

The initial source of salmonellosis is frequently not identified. Potential sources of infection include consumption of contaminated food or water, contact with contaminated environmental surfaces, equipment or handlers, aerosol exposure and direct contact with shedding animals. Mares may be inapparent shedders and shed the bacteria at parturition, infecting newborn foals (Traub-Dargatz et al., 2007). Following oral infection, invasion of the enterocytes takes place, with progression into the mesenteric lymph nodes, from where Salmonella enter the blood circulation via efferent lymph vessels (Clarke et al., 1993).

Salmonella spp. infections can present in varying clinical forms in horses, such as asymptomatic infections, fever, anorexia and depression, severe acute diarrhea, acute septicemia or a combination of diarrhea and septicemia.

Septicemia is more common in foals and weanlings than adults, and sequelae, such as polyarthritis, osteomyelitis, omphalitis and meningoenchephalitis can be seen (Niel et al., 2010; Smith, 1981). However, neonatal septicemia is usually associated with bacteria other than *Salmonella* spp., with *Enterococcus* reported as the most common isolate (Hollis et al., 2008; Wilson et al., 1989).

The most commonly documented bacterial isolate from abdominal abscesses in foals is *Rhodococcus equi* (Nay et al., 1996; Valdes et al., 2005; Reuss et al., 2009). In adult horses, *Streptococcus* is a common cause of abdominal abscesses; however, this does not appear to be documented in foals (Mair et al., 2011; Arnold et al., 2012; Berlin et al., 2013).

Treatment options for abdominal abscesses include long-term antimicrobials or surgery, including excision, drainage and lavage (Prades et al. 1989; Elce, 2006). However, the prognosis of survival for horses with intra-abdominal abscesses has been reported as guarded, with survival rates between 26% to 71% for discharge, and 19% for long-term survival (Rumbaugh et al., 1978; Pusterla et al., 2007; Arnold et al., 2012).

The foal in this case report did not respond well to the antibiotic treatment, although the antibiotic regime was changed several times during the treatment period, and despite the fact that no resistance to the antibiotics administered was recorded. Given the large size and encapsulation of the abscess, it is possible that an insufficient penetration of the antibiotic took place. Additionally, the virulence plasmids found in *Salmonella* spp. permit them to survive and multiply within phagocytic cells, and the intracellular location protects against many antimicrobial agents (Hirsh, 2004). Necrotic bone and vascular alterations associated with ischemia also protect bacteria from the host's defense mechanisms and affect penetration of systemic antimicrobials. The clinical findings, lymphocytosis and increased SAA consistent with septic inflammation and tissue damage were supportive of the diagnosis. Radiography confirmed the presence of osteomyelitis seen as lytic areas in multiple metaphyseal and physeal regions. Such bony lesions may not always be evident radiographically in the initial stages of infection, and may take weeks to be detected (Wegener et al., 1991). It is therefore important to

note that the absence of radiographic findings does not exclude the presence of infection.

Ultrasonography was useful in assessing and determining the extent and involved structures of the abscess within the soft tissues of the left hind limb. Ultrasound of the abdominal cavity would most likely have been useful in detecting the intra-abdominal abscess. However, a fluid filled colon and cecum were the only findings detected at the time of presentation, and at the time the femoral fracture was detected, further imaging was not elected due to the poor prognosis of this finding. Additionally, as described, abdominal abscessation as a sequela to Salmonella infection in foals is an unreported finding.

In comparison to x-rays, CT provides three-dimensional volume from a large series of two-dimensional radiographic images taken around a single axis of rotation, and is useful for diagnosis as well as surgical planning. In the present case, the CT provided more information regarding the extent and severity of the bony lesions than to the radiographs, and correlated well with the pathologic findings. Additionally, both the abdominal abscess and the major fistula tracts were detected.

In dogs weighing greater than 25 kg, CT detected more clinically significant lesions than ultrasound (Fields et al., 2012). Similar results have also been found in human medicine (van Randen et al., 2011).

Contrary to adult horses, thoracic and abdominal CT imaging of the foal is possible due to their small size, and should be considered as an additional imaging option in cases with suspicion of pathology of these regions.

In the present case, the multifocal limb involvement and associated findings within the gastrointestinal tract suggest a hematogenous inoculation of the bone. *Rhodococcus equi* should be considered a major differential in cases of polyarthritis and abscess formation; however, given the *Salmonella* spp. isolate and the lack of respiratory disease, it was less likely in the current case report.

The pathogen in this case report was a very aggressive form of Salmonella. Preventive measures are important, such as good hygiene rules in stables with multiple foals and consistent assessment of the immune transfer of foals after birth. Additionally, a good immune status in the dam is also important to prevent problems in foals.

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EMERITUS: UITGEDIEND RENPAARD

In het oude Rome werden renpaarden buitengewoon hoog gewaardeerd en met respect behandeld. Stokslagen waren voorbehouden voor ezels, muilezels en muildieren. Paarden die niet langer meekonden, kregen op staatskosten een verzorgde oude dag. Ze werden ‘emeritus’ (uitgediend) genoemd. Later werd dat een betiteling van gepensioneerde militairen. Bij ons is dat nu een officiële titel voorbehouden aan gepensioneerde docenten en hoogleraren met een volledig voltooide loopbaan, vanaf 65 jaar. In Nederland is de titel ook in gebruik voor predikanten ‘op rust’. De betekenis van de term werd mede beïnvloed door bewuste contaminatie met ‘eminent’ (uitstekend).

Luc Devriese

Cytologie bij zangvogels: een nuttige diagnostische tool

Cytology in Passeriformes: a useful diagnostic tool

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SAMENVATTING

Ziekteproblemen bij in groep gehouden zangvogels zijn vaak van infectieuze aard. De waargenomen klinische klachten zijn echter dikwijls dezelfde en voornamelijk aspecifiek. Cytologisch onderzoek van de organen na post-mortemonderzoek zijn desgevallend van onschatbare diagnostische waarde. In dit artikel worden de basisprincipes van de cytologische staalnametechniek van organen en de beoordelingswijze van een cytologisch preparaat beschreven. Verder wordt een overzicht gegeven van belangrijke infectieuze aandoeningen bij zangvogels, waarvan met behulp van cytologisch onderzoek een definitieve of waarschijnlijke, etiologische diagnose gesteld kan worden. Bij deze kan dit overzicht een nuttige leidraad zijn voor elke dierenarts met zangvogels in zijn of haar patiëntenbestand.

ABSTRACT

Disease outbreaks in Passeriformes housed in group are often of infectious origin. Clinical signs observed are often similar and non-specific. In these cases, cytology is an invaluable tool for developing a presumptive or definitive diagnosis that can guide disease management decisions within a flock. In the first part of this review, the basic principles of cytological sampling techniques and the evaluation of the cytological findings are described. In the second part, an overview of important infectious diseases in Passeriformes is given, in which cytology may lead to a definitive or presumptive diagnosis.

INLEIDING

Bij zangvogels die in groep worden gehouden zijn ziekteproblemen dikwijls van infectieuze aard. Klinische klachten die desgevallend worden waargenomen zijn echter vaak dezelfde en aspecifiek. In vele gevallen wordt melding gemaakt van plotse sterfte zonder klinische symptomen of sterfte voorafgegaan door een korte periode van dik zitten, anorexie en vermageren. Af en toe wordt ook dyspneu of afwijkende mest vastgesteld. Wegens de kleine omvang van zangvogels is staalname bij een levend dier vaak beperkt. Wanneer sterfte wordt waargenomen, is post-mortemonderzoek met cytologisch onderzoek van de organen van onschatbare diagnostische waarde. Het morfologisch detail van weefselcellen en vooral van bepaalde infectieuze agentia is bij cytologie vaak duidelijker dan bij histologie. Het is daarom een onmisbare diagnostische tool om bacteriële, parasitaire, gist-, schimmel- en virale infecties aan te tonen. Bovendien is cytologie een snelle, goedkope en eenvoudige techniek die gemakkelijk door de clinicus in praktijk kan gebracht worden en bij een groot deel van deze ziekteproblemen kan leiden tot een definitieve of waarschijnlijkheidsdiagnose. Hierdoor kan snel gestart worden met een gerichte therapie bij de overige zieke vogels in de groep, eventueel in afwachting van verder bacteriologisch, histologisch en PCR-onderzoek.

In het eerste deel van dit artikel worden kort de basisprincipes van de staalnametechniek en de beoordeling van een cytologisch preparaat beschreven. In het tweede deel wordt op basis van de waargenomen ziekte aan de Afdeling voor Pluimvee, Bijzondere Gezelschapsdieren, Wildlevende dieren en Proefdieren van de Faculteit Diergeneeskunde (UGent), een overzicht gegeven van belangrijke infectieuze aandoeningen bij zangvogels, waarvan met behulp van cytologisch onderzoek een definitieve of waarschijn-

lijke, etiologische diagnose gesteld kan worden. Hierbij wordt elke aandoening geïllustreerd met een karakteristiek cytologiebeeld.

TECHNIEK

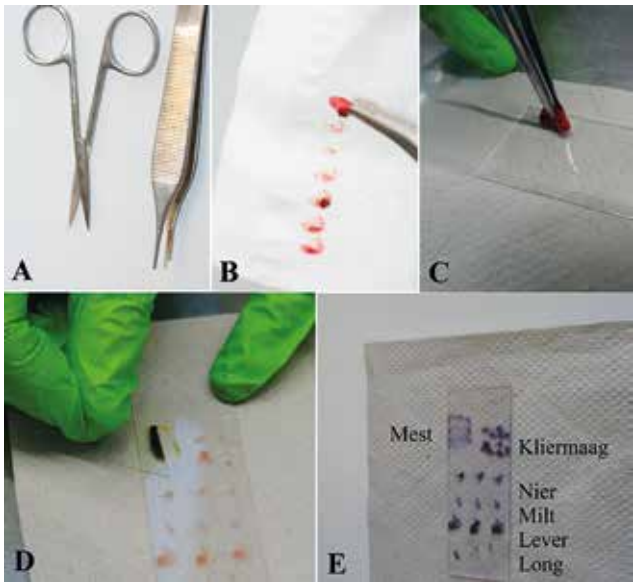
De benodigdheden voor cytologisch onderzoek zijn een schaar of bistouri en pincet, papieren tissues, microscopieglaasjes, dekglaasjes, een snelle bloedkleuring en een microscoop. Hoewel de procedure eenvoudig is, is het nemen van kwalitatief goede stalen van primordiaal belang. Stalen worden het beste genomen van vers gestorven dieren. Wanneer meerdere dieren in een groep zijn aangetast, kan eventueel een ziek dier geëuthanaseerd worden voor post-mortemonderzoek. Standaard worden door de auteurs bij post-mortemonderzoek orgaanafdrukken genomen van long, lever, milt en nier. Verder worden uitstrijkjes genomen van de kliermaagmucosa en darminhoud om de aanwezigheid van de pathogene flora te evalueren. Wanneer andere organen macroscopisch afwijkend zijn, kunnen bijkomende afdrukken van deze organen worden genomen.

Staalname

Het doel van de techniek is een "monolayer" van cellen te verkrijgen, waarbij celbeschadiging minimaal is.

Orgaanafdrukken

Voor het maken van een orgaanafdruk wordt met een vers ingesneden weefseloppervlakte gestart. Wanneer macroscopisch letsels zichtbaar zijn, bijvoorbeeld necrosehaarden en granulomen, is het aan te raden een staal te nemen doorheen het letsel, met ook intact weefsel op het afdrukpreparaat. Stalen van grote necrosehaarden zijn bijvoorbeeld in het centrum niet diagnostisch; aan de periferie zullen de cellen nog intact zijn. Om contaminatie van het snij-oppervlak te vermijden, is het belangrijk om bij elke staalname proper snijmateriaal (schaar of bistouri) te hanteren. Vervolgens wordt het snij-oppervlak verscheidene malen voorzichtig afgedrukt op een propere papieren doek om overmatig vocht en bloed te verwijderen. Dit is belangrijk aangezien door de aanwezigheid van overmatig perifeer bloed de diagnostische cellen worden verdund en gemaskeerd (Dorrestein, 2008). Vervolgens worden afdrukken gemaakt op een microscopieglaasje. Bij het afdrukken is het belangrijk niet teveel druk op de weefselstukjes uit te oefenen, aangezien dit kan leiden tot het stukdrukken van de weefselcellen. Per orgaan worden verschillende afdrukken gemaakt. Hierbij mogen de verschillende afdrukken elkaar niet overlappen; dit om het verkrijgen van een monolayer niet te verhinderen. Afdrukken van verschillende organen kunnen verzameld worden op één microscopieglaasje. De staalname wordt geïllustreerd in Figuur 1.



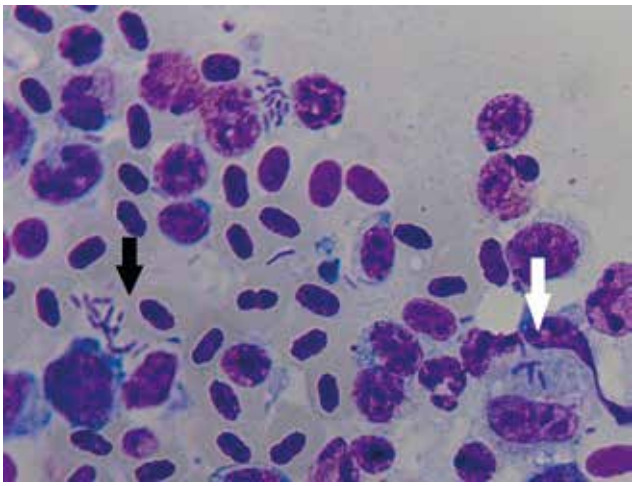
Figuur 1. Staalnametechniek. A. Start met proper materiaal. B. Het snijvlak van het orgaan wordt eerst verscheidene malen voorzichtig afgedrukt op een propere papieren doek om overmatig vocht en bloed te verwijderen. C. Vervolgens worden orgaanafdrukken gemaakt op een microscopieglaasje. Per orgaan worden verschillende afdrukken gemaakt. De afdrukken mogen elkaar niet overlappen. D. Voor het maken van een direct uitstrijkje wordt de mestinhoud met de rand van een dekglaasje over een hoek van 45° zo dun mogelijk naar de andere rand van het microscopieglaasje uitgesmeerd. E. Na drogen worden de stalen gekleurd. Door de auteurs wordt standaard een Hemacolor®-kleuring gebruikt. Afdrukken van verschillende organen kunnen verzameld worden op één microscopieglaasje.

Directe uitstrijkjes

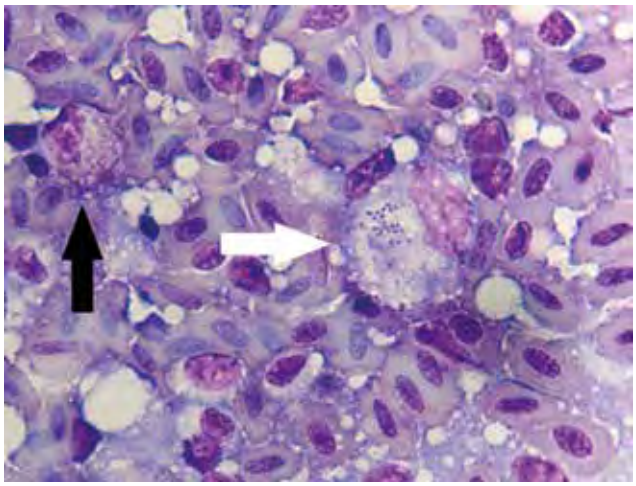
Voor het maken van een kliermaaguitstrijkje wordt de mucosale zijde van de kliermaag rechtstreeks afgedrukt op een microscopieglaasje. Of men kan met een dekglaasje een afkrabsel nemen van de slijmlaag en deze vervolgens uitstrijken op een microscopieglaasje. Ook voor het maken van een mestuitstrijkje wordt met een dekglaasje een afkrabsel genomen van de darminhoud. Met de rand van een dekglaasje over een hoek van 45° wordt de mestinhoud voorzichtig en zo dun mogelijk naar de andere rand van het microscopieglaasje uitgesmeerd (Figuur 1D).

Kleuringen

Alvorens te kleuren is het belangrijk de stalen goed te drogen om optimaal celdetail en celadhesie te garanderen. Dit kan door het preparaat te drogen aan de lucht of door het preparaat even op de radiator te leggen. Het gebruik van een bunsenbrander of haardroger wordt echter afgeraden.



Figuur 2. Sepsisbeeld van de milt van een kanarie. Aanwezigheid van talrijke, aankleurbare, bacteriële staven zowel extracellulair (zwarte pijl) als gefagocyteerd door macrofagen (witte pijl). Gerangschikt van hoge naar lage prevalentie kan differentiaal diagnostisch gedacht worden aan een infectie met *Yersinia pseudotuberculosis*, *Salmonella spp.*, *Escherichia coli* en *Listeria monocytogenesis.* (Hemacolor®, 1000X).



Figuur 3. *Enterococcus faecalis*-infectie. Septisch ontstekingsbeeld in een afdruk van de lever van een kanarie met infiltratie van heterofielen (zwarte pijl) en macrofagen met gefagocyteerde kokken (witte pijl). (Hemacolor®, 1000X).

Voor algemene cytologie wordt gebruik gemaakt van romanowsky-typekleuringen, zoals wright's en may-grünwald-giemsakleuringen. Commerciële benamingen voor deze kleuringen zijn onder andere Diff Quick® en Hemacolor®. Bij deze kleuringen wordt de celmorfologie duidelijk doordat basofiele en eosinofiele elementen met elkaar in contrast worden gebracht (Campbell en Ellis, 2007a). Deze kleuringsprocedures zijn eenvoudig, snel (< 20 seconden) en geven een goede kleurkwaliteit. Door de auteurs wordt standaard een Hemacolor®-kleuring gebruikt (Figuur 1E), zo ook in de cytologische illustraties verder in dit artikel. Indien gewenst kunnen ook bijkomende kleuringen gebruikt worden, zoals zhiel-neelsenkleuring voor het aantonen van *Mycobacterium spp.* of een STAMP-kleuring voor het aantonen van *Chlamydia spp.*. Het kleuren gebeurt volgens de gebruiksaanwijzing van de specifieke kleuring.

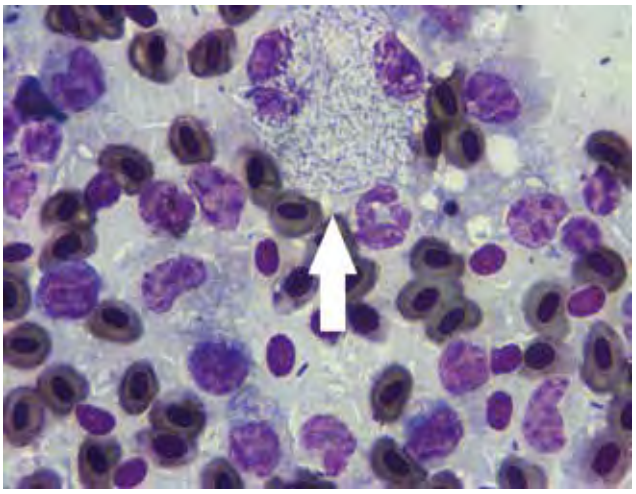
MICROSCOPISCHE BEOORDELING

Een eerste oriënterend microscopisch onderzoek gebeurt op een lage vergroting (X100). Op deze vergroting kunnen weefsels geïdentificeerd, de cellulariteit van het preparaat beoordeeld en reeds eventuele grote infectieuze pathogenen, bijvoorbeeld *Macrorhabdus ornithogaster,* schimmelhyfen, herkend worden. Ook wordt bepaald welke zone het beste bekeken wordt met een hoge vergroting (immersie-olie, X1000). Interpretaties gebeuren het beste ter hoogte van zones met een monolayer (Dorrestein, 2008; Campbell en Ellis, 2007a).

Op hoge vergroting (immersie-olie, X1000) wordt het preparaat verder in detail beoordeeld. Er wordt onder andere gekeken naar celstructuur, bacteriën of andere kleine structuren, zoals celinclusies (Dorrestein, 2008; Campbell en Ellis, 2007a). Zowel bij vergroting X100 als X1000 worden telkens verschillende velden geïnspecteerd om te kunnen nagaan wat er zich in het orgaan afspeelt.

Op hoge vergroting worden eerst de orgaancellen bekeken. Er wordt nagegaan of deze cellen een normale of abnormale morfologie vertonen, zoals degeneratie, necrose, inclusies en neoplasie. Ook worden het aantal aanwezige rode bloedcellen en hun morfologie beoordeeld: een verhoogd aantal erytrocyten, een verhoogd aantal immature erytrocyten of de aanwezigheid van inclusies kunnen respectievelijk wijzen op orgaanstuwing, regeneratieve anemie en bloedparasieten (Campbell en Ellis, 2007b).

Een verhoogd aantal leukocyten is indicatief voor inflammatie. De belangrijkste ontstekingscellen bij vogels zijn heterofielen, lymfocyten, plasmacellen en macrofagen (Campbell, 1994a; Mitchell en Johns, 2008). Bij een acuut ontstekingsbeeld wordt een infiltraat van voornamelijk heterofielen (i.e. > 70% van de ontstekingscellen) waargenomen (Montali, 1988). Ook stressbeeld kan leiden tot heterofieleninfiltratie (Gildersleeve et al., 1987). Aangezien macrofagen snel, i.e. reeds na enkele uren, migreren naar een ontstekingshaard, wordt een gemengd ontstekingsinfiltraat met heterofielen en mononucleaire leukocyten (macrofagen, plasmacellen en lymfocyten) bij vogels het vaakst waargenomen (Montali, 1988). Aldus impliceert een dominant macrofageninfiltraat (> 50%) niet noodzakelijk een chronische respons, doch kan het bij vogels suggestief zijn voor welbepaalde ziekten, zoals tuberculose, chlamydiose, salmonellose of mycotische infecties (Jortner en Adams, 1971; Awandhiya, 1981). Ook bepaalde neoplastische aandoeningen kunnen leiden tot een verhoogd aantal leukocyten samen met morfologische veranderingen, bijvoorbeeld lymfoma. Aangezien in dit artikel gefocust wordt op infectieziekten, wordt hier niet dieper op ingegaan.



Figuur 4. *Mycobacterium*-infectie. Septisch ontstekingsbeeld in een afdruk van de milt van een kanarie met aanwezigheid van talrijke, niet-aankleurbare, bacteriele staven zowel extracellulair als gefagocyteerd door macrofagen (witte pijl). (Hemacolor®, 1000X).

Tenslotte wordt het preparaat gecontroleerd op de aanwezigheid van pathogenen, zowel intracellulair als extracellulair, i.e. bacteriën, schimmels, gisten, virale inclusies, parasieten.

CYTOLOGISCHE KARAKTERISTIEKEN VAN INFECTIEUZE PATHOGENEN

In de periode 2013-2017 werden aan de Afdeling voor Pluimvee, Bijzondere Gezelschapsdieren, Wildlevende Dieren en Proefdieren van de Faculteit Diergeneeskunde (UGent) 224 autopsies uitgevoerd bij zangvogels. De waargenomen klachten waren steeds aspecifiek, namelijk sterfte, al dan niet voorafgegaan door een korte periode van minder eten en dik zitten. Soms werd ook dyspneu of afwijkende mest waargenomen. In 72% van deze autopsiegevallen kon de definitieve diagnose of waarschijnlijkheidsdiagnose gesteld worden door het aantonen van het etiologische agens met behulp van cytologisch onderzoek. Hierbij dient opgemerkt te worden dat in 42% van de cases een multifactoriële problematiek werd vastgesteld. Het meest frequent werden co-infecties met *Macrorhabdus ornithogaster*, atoxoplasmose en/of bacteriele sepsis waargenomen. Hieronder wordt een overzicht gegeven van belangrijke infectieuze aandoeningen waarbij cytologisch onderzoek kan leiden tot een etio-logische diagnose, de cytologische karakteristieken die desgevallend worden waargenomen, alsook de waargenomen prevalentie door de auteurs.

Cytologie geassocieerd met bacteriële aandoeningen

Sepsis

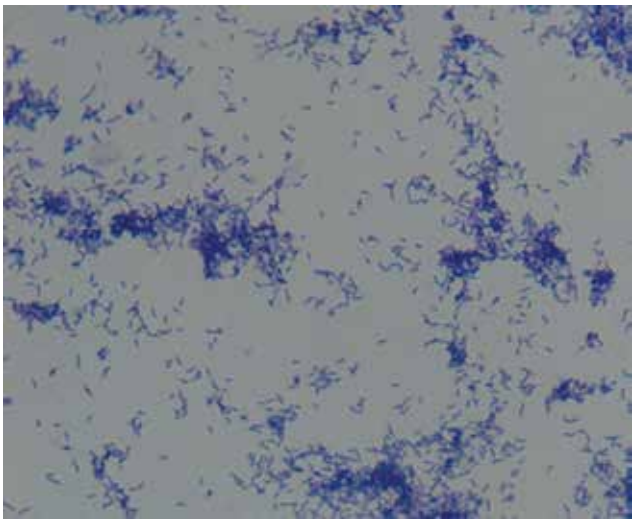
In 43% van de gevallen was de sterfte bij zangvogels het gevolg van bacteriële sepsis. Hiervan werd 23% veroorzaakt door een infectie met *Yersinia pseudotuberculosis*, 6% door *Salmonella spp.*, 5% door *Escherichia coli*, 4% door *Mycobacterium spp.* en 3% door een infectie met *Enterococcus faecalis.* Sporadisch werd sepsis ten gevolge van *Listeria monocytogenes* (1%), *Pasteurella multocida* (0,5%) of *Staphylococcus spp.* (0,5%) vastgesteld.

Bij rechtstreekse afdrukken van diverse organen worden na snelle bloedkleuring (Hemacolor®) een verhoogd aantal ontstekingscellen, voornamelijk macrofagen en lymfocyten, en een zeer talrijke aanwezigheid van bacteriën waargenomen. Deze bacteriën zijn soms opvallend aanwezig in het cytoplasma van macrofagen (fagocytose). *Yersinia pseudotuberculosis*, *Salmonella spp., Escherichia coli, Pasteurella multocida* en *Listeria monocytogenesis* zijn alle staafjes die sterk basofiel aankleuren na Hemacolor® (Figuur 2). Enterokokken en Stafylokokken zijn op cytologie waar te nemen als basofiele kokvormige bacteriën (Figuur 3). Bij infectie met *Mycobacterium spp.* worden talrijke ongekleurde aftekeningen van bacteriële staafjes gezien (Figuur 4).

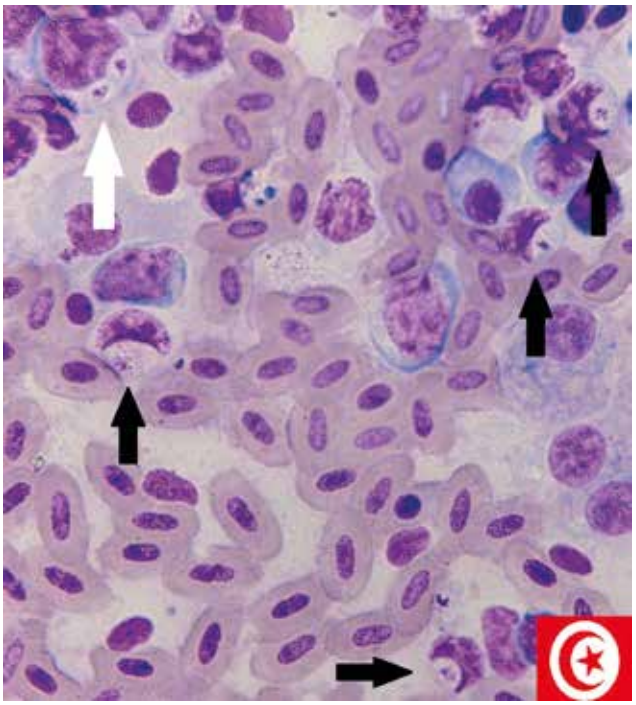
Infecties met *Mycobacterium spp*., *Escherichia coli* en *Enterococcus faecalis* leiden bij zangvogels vaak tot acute sepsis (Eatwell, 2008; Martel en Pasmans, 2013). Op lijkschouwing valt een erge lever- en miltzwelling op. Infecties met *Yersinia pseudotuberculosis*, *Salmonella spp*. en *Listeria monocytogenesis* kennen zowel een acuut als chronisch ziekteverloop (Eatwell, 2008; Martel en Pasmans, 2013). Op autopsie worden speldenkop-grote granulomen of necrosehaarden gevonden bij een vergrote lever en milt. De milt is vaak het ergste aangetast. Op basis van de waargenomen prevalentie van deze infecties, de letsels ter hoogte van de milt en lever (met of zonder granuloomvorming) en de morfologie van de bacteriele populatie op cytologie kan reeds een waarschijnlijkheidsdiagnose worden gesteld. Deze kan vervolgens bevestigd worden door middel van bacteriële cultuur of in het geval van *Mycobacterium* met een ziehl-neelsenkleuring, eventueel aangevuld met PCR-onderzoek. In afwachting van het resultaat kan echter al gestart worden met een gerichte therapie bij de overige vogels.

Campylobacteriose

Campylobacteriose werd door de auteurs in 6% van de gevallen vastgesteld. Deze darminfectie, veroorzaakt door *Campylobacter jejuni*, komt voornamelijk voor bij prachtvinken. Bij verminderde afweer kan een infectie met *Campylobacter jejuni* leiden tot klinische symptomen, waarbij dik zitten, waterige, gele mest of geelachtige meststaafjes en sterfte worden waargenomen (Eatwell, 2008; Martel en Pasmans, 2008). Op een uitstrijkje van de darminhoud van een levend of vers gestorven dier wordt na Hemacolor®-kleuring een homogene populatie van bacteriën met *Campylobacter*-morfologie waargenomen (komma-, S- of V-vormig) (Figuur 5). Vaak is de ziekte multi-



Figuur 5. Campylobacteriose. Uitstrijkje van de darminhoud van een gouldsamadine met een monomorfe populatie van bacteriën met *Campylobacter*-morfologie: V-vormig of 'meeuwtjes'. (Hemacolor®, 1000X).



Figuur 6. Atoxoplasmose in een afdruk van de lever van een goudvink. De parasieten zijn te zien als intracytoplasmatische inclusies in mononucleairen. Meestal is er één parasitaire inclusie per mononucleair te zien (zwarte pijlen), te vergelijken met het uitzicht van de vlag van Tunesië (insprong rechts onder). Soms zijn er ook meerdere atoxoplasma-inclusies per aangetaste cel waar te nemen (witte pijl). (Hemacolor®, 1000X).

factorieel en komt ze samen voor met flagellaten, zoals *Cochlosoma* en coccidiën. Aldus wordt geadviseerd om ook parasitologisch mestonderzoek uit te voeren. Voor het aantonen van flagellaten dient deze mest zeer vers te zijn.

Cytologie geassocieerd met parasitaire aandoeningen

Atoxoplasmose

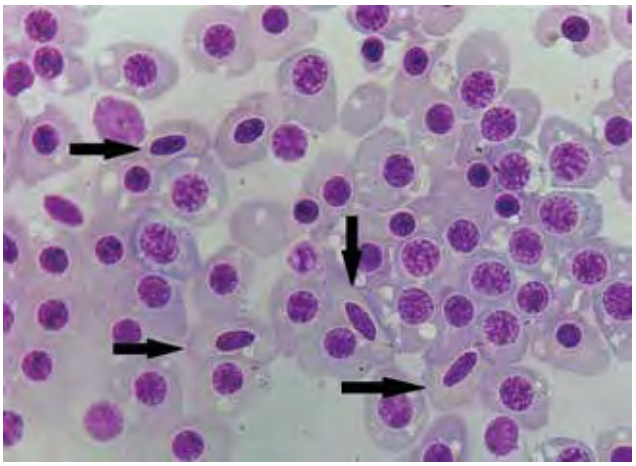
Atoxoplasmose werd door de auteurs in 23% van de gevallen vastgesteld. De symptomen van klinische atoxoplasmose zijn atypisch en zijn meestal beperkt tot dik zitten en dyspneu. De mortaliteit kan oplopen tot 80%. De ziekte zou veroorzaakt worden door een extra-intestinaal stadium van *Isospora spp.*. Omdat na de acute fase slechts een beperkt aantal oöcysten uitgescheiden wordt, is het ante mortem moeilijk om de diagnose te stellen (Eatwell, 2008). De diagnose wordt dan ook meestal gesteld op lijkschouwing via cytologisch onderzoek. Long en lever zijn vaak het ergste aangetast. De parasieten zijn te zien als karakteristieke intracytoplasmatische inclusies in mononucleaire cellen. De auteurs vergelijken het cytologisch beeld van de aangetaste mononucleaire cellen met het uitzicht van de Tunesische vlag. De kern van de mononucleaire cel wordt namelijk door de parasiet 'opzij geduwd' en krijgt hierdoor de vorm van een 'krimpende maan'. De kern van de parasiet kan met de ster vergeleken worden (Figuur 6).

Rode vogelmijt

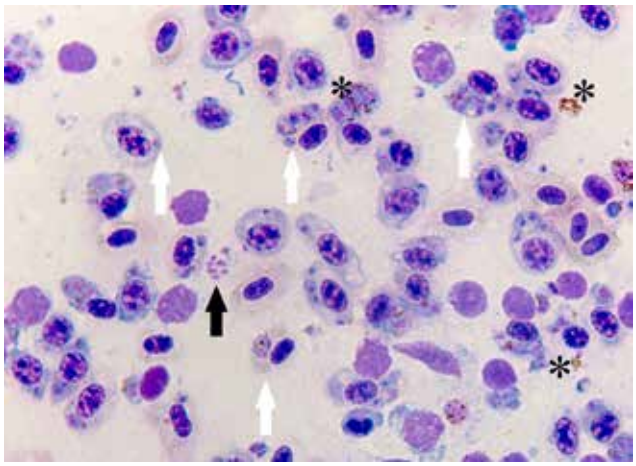
Een frequent voorkomende ectoparasiet bij zangvogels is *Dermanyssus gallinae*. Aangezien deze bloedzuigende mijten enkel 's nachts op de dieren parasiteren en zich overdag verstoppen in spleten, worden ze vaak niet door de eigenaar waargenomen. De vogels zijn 's nachts onrustig en een zware infestatie leidt tot anemie en zelfs sterfte. Meestal zijn de parasieten op lijkschouwing niet terug te vinden; indien toch, dan is dit meestal indicatief voor ernstige mijteninfestaties in de volière. Op een afdrukpreparaat van de long van een gestorven dier of op een bloeduitstrijkje van een levend dier, wordt echter een typisch beeld van regeneratieve anemie met verhoogde aanwezigheid (> 10%) van immature erytrocyten waargenomen (Campbell en Ellis, 2007b). De normale mature erytrocyt bij vogels is ovaal met een centraal gepositioneerde ovale kern. Het cytoplasma kleurt oranje-rozig en de kern is gecondenseerd en donker paars (Campbell, 1994b). Immature rode bloedcellen zijn rond, polychromatisch met een basofiel cytoplasma en een minder condense, rondere kern (Campbell, 1994b; Mitchell en Johns, 2008) (Figuur 7). Een beeld van regeneratieve anemie zonder aanwijsbare oorzaak, bijvoorbeeld gastro-intestinale parasitose, bloedparasieten, etc. werd in 8% van de aangeboden cases vastgesteld. In deze gevallen wordt geadviseerd om de volière te controleren op de aanwezigheid van rode vogelmijt.

Malaria

Eenmaal werd door de auteurs een *Plasmodium-*



Figuur 7. Regeneratieve anemie. Op deze afdruk van de long van een gouldsamadine zijn slechts enkel mature erytrocyten, i. e. ovaal van vorm en met condense ovale kern, aanwezig (zwarte pijlen). De meeste erytrocyten zijn immatuur, waardoor nog rond van vorm en polychromatisch met basofiele cytoplasma's en minder condenseerde, nog ronde kernen. (Hemacolor®, 1000X).



Figuur 8. *Plasmodium*-infectie. Massale aanwezigheid van immature, ronde, polychromatische erytrocyten als gevolg van een regeneratieve respons in een afdruk van de long van een kanarie. Verschillende ontwikkelingsstadia van *Plasmodium* zijn talrijk aanwezig zowel in het cytoplasma van de erytrocyten (witte pijlen) als extracellulair (zwarte pijl). Ook zijn er enkele klonters hemozoïne (*) waarneembaar. (Hemacolor®, 1000X).

infectie vastgesteld (0,5% van de gevallen). Op een bloeduitstrijkje van een levend dier of orgaanafdrukje van de long van een gestorven dier worden na snelle bloedkleuring (Hemacolor®) een beeld van regeneratieve anemie, alsook de parasieten in de bloedcellen gezien (Figuur 8). Gametocyten, schizonten en trofozoïeten van *Plasmodium spp.* kunnen bij vogels worden gevonden in erytrocyten, trombocyten en leukocyten (Campbell en Ellis, 2007b). Ook kunnen bruine pigment fracties worden waargenomen. Dit is hemozoïne geproduceerd door *Plasmodium* na digestie van hemoglobine.

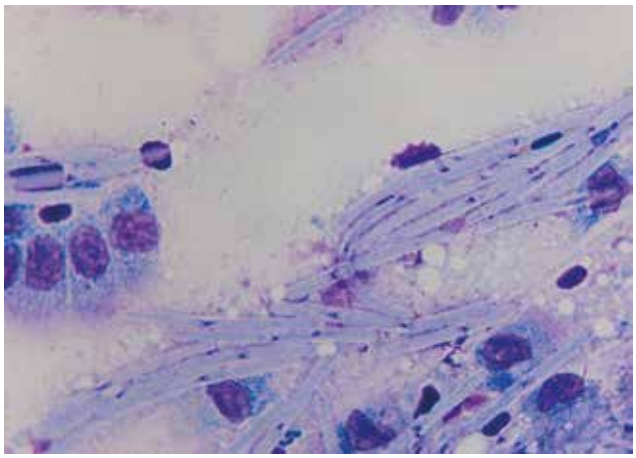
Cytologie geassocieerd met mycotische aandoeningen

Macrorhabdus ornithogaster

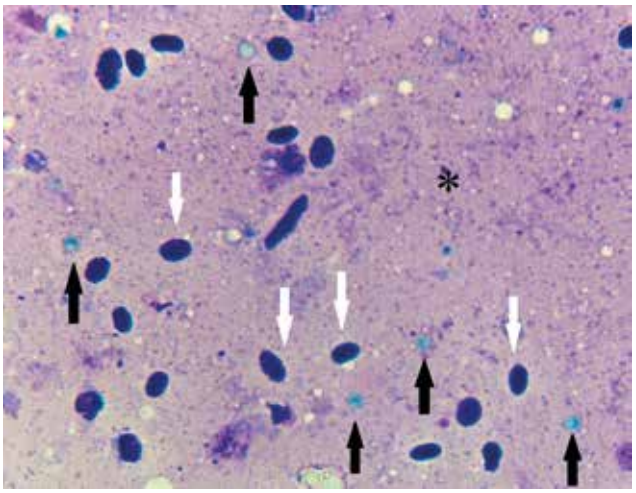
Deze vaak voorkomende gistinfectie van de kliermaag werd in 40% van de gevallen door de auteurs vastgesteld. Een sterke toename van deze gisten in de kliermaag leidt tot het afsluiten van de mucosakliertjes, waardoor de secretie van de HCl en pepsine wordt geremd. Er worden onverteerde zaden in de feces waargenomen, de algemene conditie van de vogel verslechtert en er treden gewichtsverlies en sterfte op (Davies, 2008; Martel en Pasmans, 2013). Op autopsie wordt een gedilateerde, dikwandige kliermaag met vaak witte mucus opstapeling ter hoogte van het mucosaal oppervlak gezien. De diagnose kan eenvoudig gesteld worden via cytologisch onderzoek van de kliermaag. Op een afkrabsel van de mucosae worden na snelle bloedkleuring bij klinische gevallen grote aantallen gisten vastgesteld. Deze gisten hebben het karakteristiek uitzicht van grote (> 20 µm) licht basofiele staven gelijkend op hyfen vaak afgelijnd door middel van een witte rand, het zogenaamde halo-effect (Figuur 9). Bij klinisch duidelijke gevallen kunnen de gisten zodanig sterk vermenigvuldigen, dat zij in grote aantallen het darmkanaal passeren. Desgevallend is er een redelijke kans om de gisten met behulp van een snelle bloedkleuring ook aan te tonen in een fecesuitstrijkje van nog levende dieren.

Aspergillose

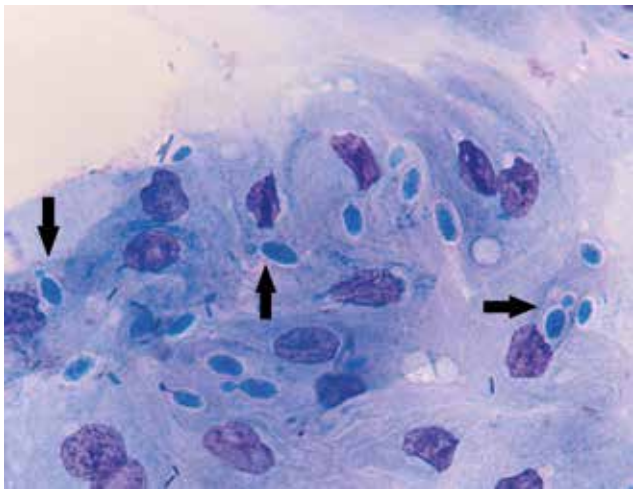
Een *Aspergillus fumigatus*-infectie bij zangvogels geeft aanleiding tot dyspneu zonder ademgeluiden. Meestal is slechts een beperkt percentage van de dieren aangetast en is er weinig sterfte. Predisponerende factoren zijn een vochtig en warm klimaat, onhygiënische omstandigheden, overbezetting en beschimmeld



Figuur 9. *Macrorhabdus ornithogaster*-infectie. Op een afkrabsel van de kliermaagmucosae van een barmsijs worden de gisten als grote, licht basofiele staven gezien. Bemerk ook het halo-effect zichtbaar als een wit aura rondom de gist. (Hemacolor®, 1000X).



Figuur 10. Aspergillose. Aanwezigheid van talrijke schimmelsporen (zwarte pijlen) in de long van een zebravink met oedeem (*) en gelyseerde erytrocyten (witte pijlen). (Hemacolor®, 1000X).



Figuur 11. Candidiase. Aanwezigheid van talrijke ovale gisten op een uitstrijkje van de darminhoud van een kanarie. Bemerk de typische knopvorming (zwarte pijlen) alsook het halo-effect, zichtbaar als een wit aura rondom de gist. (Hemacolor®, 1000X).

voeder of beschimmeld neststrooisel (Macwhirter, 1994; Martel en Pasmans, 2013). De diagnose is in vivo moeilijk te stellen en gebeurt meestal pas na lijkschouwing. Een karakteristiek letsel is granuloomvorming ter hoogte van de luchtwegen (aspergillomen). Op cytologisch onderzoek kunnen de schimmelsporen als contrasterende basofiele bolletjes worden waargenomen op afdrukpreparaten van de longen en/of luchtzakken (Figuur 10). Schimmelhyfen worden vrijwel nooit aangetroffen op cytologisch onderzoek. Aspergillose komt zelden voor bij graaneters, zoals kanaries en prachtvinken. Fruiteters en insecteneters worden vaker getroffen. Door de auteurs werd de diagnose van aspergillose in 4% van de gevallen vastgesteld.

Candida

Zowel kropcandidiase als darmcandidiase komt voor. Dieren met kropcandidiase vertonen een verminderde voederopname, braken, kropovervulling en eventueel diarree en dyspneu. Darmcandidiase wordt gekenmerkt door dik zitten, een verminderde voederopname, half vloeibare feces, maar meestal weinig sterfte. De aandoening komt voornamelijk voor bij fruit- en honingetende vogels. Daarnaast wordt de aandoening ook vastgesteld bij insecteneters, prachtvinken en recessief witte kanaries. Predisponerend zijn een onevenwichtige voeding, immunosuppressie, antibioticabehandeling en in het geval van recessief witte kanaries een vitamine A-tekort (Martel en Pasmans, 2013). Deze vogels zijn namelijk deficiënt voor de aanmaak van vitamine A uit caroteen. De diagnose kan gemakkelijk gesteld worden door het aantonen van de gisten met rechtstreekse snelle bloedkleuring (Hemacolor®) van uitstrijkjes van de darminhoud of krop (Figuur 11). Deze ovale gist vertoont knopvorming en vaak is ook een “halo” waar te nemen. Op autopsie werd candidiase door de auteurs in 2% van de gevallen waargenomen.

CONCLUSIE

Op basis van de autopsieresultaten uitgevoerd op zangvogels in de periode 2013-2017 blijkt cytologisch onderzoek na post-mortemonderzoek van onschatbare waarde. In 72% van de ingezonden cases kon met behulp van cytologie een etiologische diagnose of waarschijnlijkheidsdiagnose gesteld worden. Met behulp van de cytologische illustraties beschreven in dit overzicht kan de clinicus zich zelf bekwamen in het aflezen van cytologische preparaten om een snelle, aanvullende diagnose te bekomen wanneer hij of zij geconfronteerd wordt met groepssterfte. Dit met het oog op het starten van een gerichte therapie, in afwachting van (indien nodig) de resultaten van verder bacteriologisch, histologisch en/of PCR-onderzoek.

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AFTANDS

We kennen en respecteren het gezegde dat je een gekregen paard niet in de muil mag kijken. Een cadeautje mag je niet te kritisch beoordelen. Maar wat als je iets aftands kreeg? Ook ‘aftands’ stamt uit de paardenhandel. Het is afgeleid van de tandwisseling en meer nog de slijtage die duidt op een oude knol: die is ‘aftands’.

Luc Devriese

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Diergeneeskundige kennis in de lekenpraktijk Handschrift Bouckaert anno 1880

A veterinary manuscript dated 1880, composed and used by a family of mainly lay therapists

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SAMENVATTING

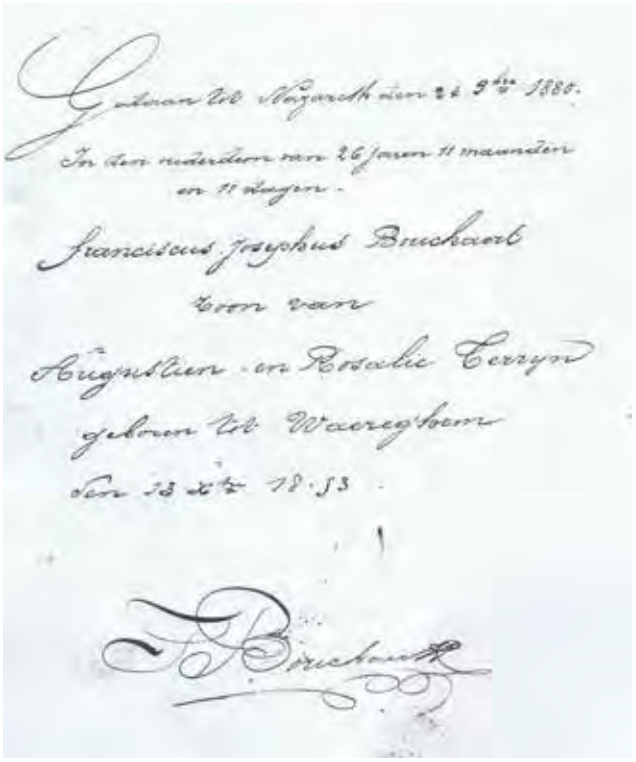
Een handschrift in 1880 opgesteld door een lid van de vooral in paarden- en dierenartsenmiddens gekende Waregemse familie Bouckaert, geeft een goed idee van de ziekteleer en geneeskunde van dieren in de praktijk van die tijd. Die was nog voor een groot gedeelte in handen van ongediplomeerde genezers, meestal in hoofdberoep hoefsmid, soms boer, of zoals Francis Bouckaert, opsteller van het hier behandelde handschrift, slachter. Deze Bouckaert blijkt goed op de hoogte geweest te zijn van de toenmalige veeartsenijkundige literatuur, wat niet belette dat hij daarnaast bezweringsformules en middeltjes opnam, gebruikt in de volksgeneeskunde. Opmerkelijk in het handschrift is ook de aandacht besteed aan duivenziekten. In een duidelijk afgescheiden gedeelte wordt een korte beschrijving gegeven van koopvernietigende gebreken en de omgang met sommige erg infectieuze veeziekten, voorbehouden aan gediplomeerde veeartsen, de 'experts vétérinaires' van die tijd. Als voorbeeld van accurate beschrijving van een ziektebeeld in het handschrift, met de nodige reserves bij de opgave van gebruikelijke behandelingen, wordt een citaat over de ziekte van Carré weergegeven.

ABSTRACT

A manuscript on veterinary knowledge and skills as practiced by laymen in previous centuries is described, using a copy dated 1880 but with parts that are probably older. It was made and owned by members of the Bouckaert family from Waregem, who were well known in the veterinary practice and horsemanship circles of that time. Some of them were among the first official veterinary surgeons and certified 'farrier-veterinarians' (maréchaux-vétérinaires) in Belgium during the nineteenth century. During successive generations, the older family fathers were also involved in the treatment of humans, while the care of animals was left to the sons. The very orderly presented contents of the manuscript show a curious mixture of veterinary science, as contained in eighteenth- and nineteenth-century textbooks, intermixed with folk remedies, including oaths and semi-religious vows. Particular attention is given to pigeon diseases. A separate chapter contains succinct descriptions of some greatly feared contagious animal diseases, which were the domain of university trained veterinary 'experts'. A text fragment on canine distemper is given as an example of accurate description of symptoms, with warnings on prognosis and futile treatment attempts.

INLEIDING

Medeauteur Jan Bouckaert, arts, schonk aan de Museumcollectie Diergeneeskundig Verleden Merelbeke een aantal werken uit de bibliotheek van zijn vader, Jean-Isidore (Henri) Bouckaert, de alom gewaardeerde eerste hoogleraar heelkunde van de huisdieren in Gent. Een uitzonderlijk stuk betreft een kopie van een volumineus manuscript getiteld *Boek der Ontleedkunde, Ziekten, Ziektekenteekenen en Ziekte-*



Figuur 1. Beginpagina van het handschrift.

behandelingen der Dieren. Het werd in 1880 opgemaakt door Franciscus Josephus Bouckaert, slachter te Nazareth (Figuur 1). Het volumineuze, zeer ordelijk gestelde stuk is vermoedelijk gebaseerd op nog oudere familieoverleveringen. Een beschrijving van het handschrift en een korte familiegeschiedenis zijn te vinden bij Bouckaert, 2006 en 2015. In deze korte bijdrage worden enkele voor de geschiedenis van de diergeneeskunde belangrijke aspecten van dit werk naar voor gebracht.

LEKENPRAKTIJK

Leken zoals de slachter Francis Bouckaert waren terdege op de hoogte van de overigens schaars voorradige veeartsenijkundige literatuur. Een tijdlang werden ze trouwens officieel erkend als 'maréchal vétérinaire', titel stammend uit de Napoleontische legerorganisatie waarin paarden een erg grote rol speelden en dierenartsen opgeleid aan de nog maar pas gestichte veeartsenijscholen schaars waren (Mammerickx, 1967 en 1997; Bols et al., 2015). Na deelname aan de campagne van Napoleon in Spanje verkreeg een Bouckaert de door de staat erkende titel 'maréchal vétérinaire'. Een andere haalde in 1856 als een van de eersten het officiële Belgische diploma van veearts aan de nieuw gestichte 'Ecole vétérinaire' van Kuregem - Brussel. Een voorvader van de auteur van het handschrift, geboren in 1753 en uitzonderlijk oud geworden (99 jaar), was volgens zijns doodsprentje 'veeartsenykundigen'. Best mogelijk dat een gedeelte van de inhoud van het 'boek' nog op diens kennis teruggaat.

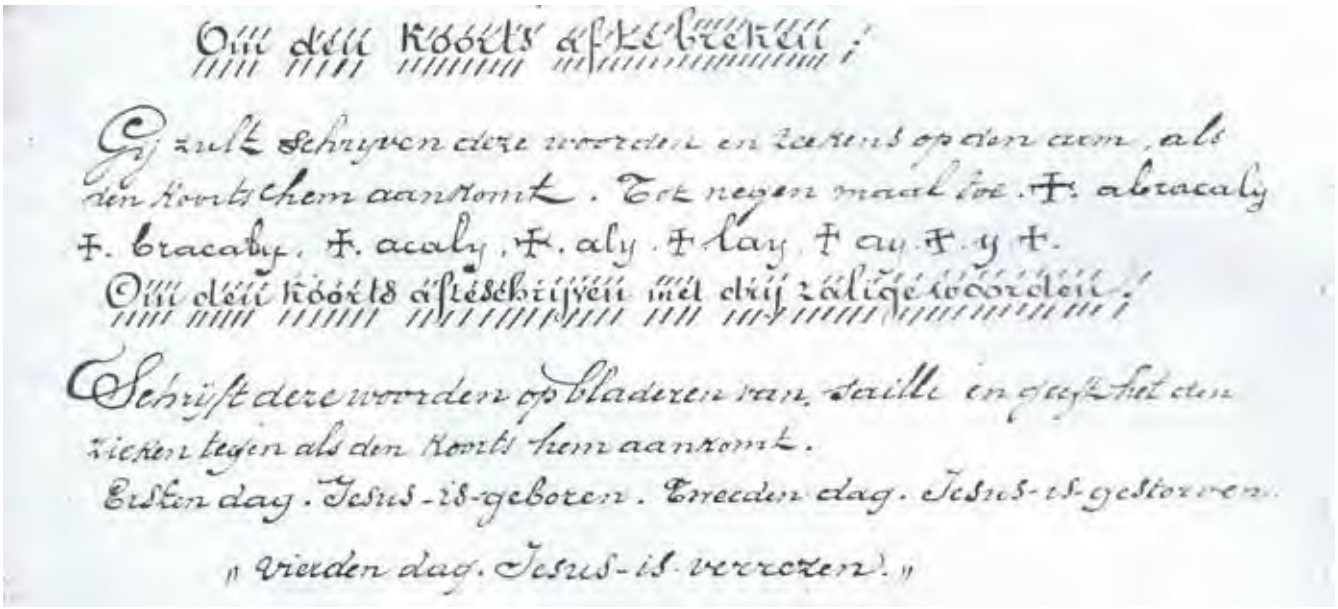
Deze vaklui mochten zich in de tweede helft van de jaren 1800 echter niet meer aan alles wagen. De beoordeling van koopvernietigende gebreken en de behandeling van sommige erg infectieuze veeziekten waren voorbehouden aan gediplomeerde veeartsen, de 'experts vétérinaires'. In het handschrift wordt dit kort beschreven (Figuur 2).

Leden van de familie Bouckaert stonden heel lang in het Waregemse bekend als 'paardenmeesters', slachters, chirurgijns en helers, vooral van dieren, maar ook van mensen. In de jaren 1800 waagden ze zich nog aan bezweringen, een praktijk die normaal voorbehouden was aan de geestelijkheid. Een fragment uit het handschrift illustreert dit (Figuur 3). Een roman van André Demedts (1941), losjes gebaseerd op de reputatie van sommige Bouckaerts, beschrijft de 'toverkunsten', onwillig uitgeoefend door een (paarden)meester die er om gesmeekt wordt door zijn zieke klanten, waaronder zelfs de dorpspastoor. Bezweringen werden uitgesproken in een verduisterde kamer bij een groot 'toverboek' – in werkelijkheid een oude uitgave van het Kruidenboek van Dodoens – omringd met kaarsen. Blijkbaar verzorgde in verschillende generaties de vader op hogere leeftijd mensen en liet hij de dieren over aan een zoon (Bouckaert, 2017). Het zwartgallige boekje van Demedts over het leven in een plattelandsgemeenschap in de Leiestreek in de jaren 1800 bevat jammer genoeg niets veterinairs.

HONDENPLAAG

Voor een beschrijving van de inhoud van het 125 pagina's tellende 'boek' wordt verwezen naar Bouckaert (2005). Om een voorbeeld te geven van wat er over sommige ziekten bekend was in het hier beschreven lekenmilieu in de 19de eeuw, volgt hieronder een fragment over de ziekte van Carré, alias de hondenplaag of jonge hondenziekte uit het Bouckaert-handschrift, anno 1880. Voordat de vaccinatie vrijwel algemeen werd toegepast, was deze zeer besmettelijke virale aandoening, verwant met de menselijke mazelen, een te duchten ziekte, nauwelijks te behandelen. Het was een gevreesde plaag, destijds alom bekend bij

Figuur 2. Aanhef van het tekstdeel over de ziekten waarmee de auteur en zijn ongediplomeerde collega's zich niet mochten bemoeien.



Figuur 3. Bezweringsformule: letterlijk abra kadabra. Saille betekent salie.

fokkers en handelaars als dé hondenziekte. Het citaat wordt woordelijk weergegeven in cursief met hier en daar een verduidelijking tussen haakjes. Enkele zinnen werden gesplitst, een enkele verplaatst.

(De hondenplaag) *bestaat in de ontsteking van het slijmvlies der maag en der longpijpen eygen aan de jonge honden, en vertoont zich in het begin door triestigheid, de vermindering van eetlust, den braaklust en de braaking, den hoest. Een vuil plakkerig slijm vloeit uit den neus en lijmt er zich rond aan. Niest dikwijls. De mond is warm en plakkend, de tong is aan de boorden rood, den dorst is groot. De oogen rood met etter beladen, het oogvlies komt troebel. Somtijds wordt het dier blind en is er opstopping en afloop* (diarree) *die het dier lijden doet. Men vindt somtijds zenuwtrekkingen die bestaan in onvrijwillige trekkingen der beenen of andere deelen des lichaams: chorée of dans van Sinte Gui* (equivalent van Sint-Vitus) *genoemd. De verlamming komt over* (wordt algemeen), *de uitteering en de dood* (volgt). *In den regelmatigen gang* (bij normaal verloop) *en wanneer er zich geen verlamming of dans verdoet* (voordoet), *duurt deze ziekte gemeenlijk 12 à 15 dagen.*

Oorzaken: *Gegronde oorzaken zijn tot nog toe niet gekend. Men schrijft dat het doorzwelgen van groote brokken vleesch of brood kan oorzaak geven alsook het eten van vuyligheden of bedorven vleesch, de landshieting* (?), *maar bovenal de besmetting.*

Behandeling*: Als de ziekte licht is, gedurende een tien à twaalftal dagen eene gezonth levenswijze is genoegzaam, maar als de ontsteeking meerder is, men moet toevlucht nemen tot het vetachtig drinken, voor alle voedsel een aftreksel van geerst, zeem, melk en water, badden* (baden) *en zachte kleysteriën* (rectale spoelingen). *Men mag ook een séton steken* (etterdracht: ontsteking opwekkende draad of lint onderhuids gestoken) *aan den nek. Als de chorée bestaat, men doet wrijvingen van terpentijn en ammoniakgeest op den rug en de benen. Men mag ook gebruik maken van den opium of afkooksel van heulbotten* (papaver).

Opmerkingen: *Ik heb zeer dikwijls een volstrekt goeden uitslag bekomen met volgende middelen. Zoohaast men de eerste teekenen bemerkt, houdt men den hond zonder voedsel. Men geeft braakwijnsteen 2 à 4 grammen in warm water opgelost en lauw-warm ingenomen. Indien den hond 7 à 10 minuten na het innemen niet braakt, geeft men hem alle 10 à 12 minuten een halve pint warm gezouten water in, totdat hij aan het braken is. Dat mag men in 2 of 3 dagen 3 à 4 maal vernieuwen, waarnaar men aan den hond warme zoete melk waarin solferbloem is opgelost te drinken geeft, 2 à 3 maal daags volgens de zwaarte van de ziekte.*

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OPRICHTING VAN EEN DIERGENEESKUNDIGE RECHTSPERSOON (DRP)

VRAAG

Sinds 1/01/18 is de diergeneeskundige rechtspersoon wettelijk in orde. We zouden graag vernemen wat het stappenplan is om hier gebruik van te maken. Dit in 2 heel concrete gevallen: Praktijk 1: rundveepraktijk, vennootschap met twee gelijkwaardige vennoten (dierenartsen) - Praktijk 2: rundveepraktijk, vennootschap met één dierenarts als vennoot-eigenaar en één dierenarts als zelfstandig medewerkster.

Wat is het stappenplan om de praktijk als rechtspersoon te zien?

ANTWOORD

Inleiding

Zonder volledigheid te claimen, probeer ik in te gaan op de vraag hoe een zogenaamde diergeneeskundige rechtspersoon, afgekort als DRP, kan worden opgericht. Dit kan evenwel niet zonder eerst in te gaan op enkele wettelijke bepalingen en op de vraag of de DRP door de wetgever gecreëerd werd om enkele praktische problemen in de dagelijkse praktijk op te lossen dan wel, in navolging van de architectenvennootschap, om dierenartsen toe te laten hun beroepsaansprakelijkheid te beperken.

Wetgevend kader

De DRP werd via de **Wet tot instelling van de Orde der dierenartsen**(1) in 2014 ingevoerd en wordt als volgt omschreven (Artikel 2, §1, 2°):

"*De diergeneeskundige rechtspersoon is de rechtspersoon die beschikt over rechtspersoonlijkheid, met een maatschappelijke zetel en bij ontstentenis ervan met een uitbatingszetel in België en die geregistreerd is bij de Kruispuntbank van Ondernemingen met een ondernemingsnummer en die aan volgende voorwaarden voldoet:*

1° alle zaakvoerders, bestuurders, leden van het directiecomité zijn natuurlijke personen die ertoe gemachtigd werden het beroep van dierenarts uit te oefenen overeenkomstig artikel 4 van de wet van 28 augustus 1991 op de uitoefening van de diergeneeskunde;

2° zijn doel en activiteit zijn beperkt tot het verlenen van diensten die behoren tot de uitoefening van het beroep van dierenarts en mogen hiermee niet onverenigbaar zijn;

3° indien hij is opgericht in de vorm van een naamloze vennootschap of een commanditaire vennootschap op aandelen, zijn zijn aandelen op naam;

4° de aandelen of deelbewijzen alsook de stemrechten zijn rechtstreeks of onrechtstreeks in het bezit van dierenartsen die de diergeneeskunde uitoefenen binnen de diergeneeskundige rechtspersoon. Nochtans kan 33 % van de aandelen en deelbewijzen in het bezit zijn van rechthebbenden van dierenartsen-vennoten, van andere dierenartsen of van diergeneeskundige rechtspersonen;

5° de diergeneeskundige rechtspersoon mag geen aandelen bezitten in andere vennootschappen of rechtspersonen waarvan het maatschappelijk doel of de activiteiten onverenigbaar kunnen zijn met de uitoefening van de diergeneeskunde.

Als de rechtspersoon niet meer voldoet aan de vereiste voorwaarden om zijn inschrijving op de lijst van de Orde te behouden, beschikt hij over een termijn van zes maanden om zich in regel te stellen met die voorwaarden. Deze termijn kan verlengd worden door de bevoegde gewestelijke raad."

Verder vermeldt de Wet van 1950(2): "*Wanneer een tuchtstraf wordt opgelegd aan een rechtspersoon, kan er een ook tuchtstraf worden opgelegd aan de natuurlijke personen die ingeschreven zijn op de lijsten van de Orde wier interventie aan de basis ligt van de feiten waarvoor de rechtspersoon een tuchtstraf kreeg.*" Dit impliceert dat een DRP zelf ook tuchtrechtelijk bestraft kan worden, net als een dierenarts natuurlijke persoon.

Aanvullend wordt in de **Wet op de uitoefening van de diergeneeskunde**(3) vermeld dat de DRP dient te zijn ingeschreven op één van de lijsten van de Orde (Artikel 4), wat aangeeft dat deze, evenwel via haar dierenartsen natuurlijke personen die zelf lid zijn van de Orde, de diergeneeskunde kan uitoefenen: "*Niemand mag de diergeneeskunde uitoefenen zonder ingeschreven te zijn als dierenarts op de lijsten van de Orde die het beroep beheren zoals bedoeld in de wet van 19 december 1950 tot instelling van de Orde der Dierenartsen of als diergeneeskundige rechtspersoon op de in dezelfde wet bedoelde lijsten van de Orde. De diergeneeskundige rechtspersonen oefenen de diergeneeskunde enkel uit via dierenartsen natuurlijke personen die gemachtigd zijn om diergeneeskundige handelingen uit te voeren.*" Dit is een buitengewone bepaling die het grote verschil met de (deontologische) rechtspersonen met diergeneeskundig doel, zoals we die al jaren kennen en die heel vaak door dierenartsen gebruikt worden om fiscale redenen en om hun privévermogen af te scheiden van dat van hun praktijk, duidelijk maakt. De DRP oefent namelijk zélf de diergeneeskunde uit. Of daaruit besloten kan worden dat de DRP alle diergeneeskundige handelingen kan stellen, dan wel diegene die specifiek voorzien zijn in de wetgeving, komt verder aan bod.

Belangrijk daarbij is te vermelden dat de vermelde dierenartsen natuurlijke personen ofwel vennoot zijn van de diergeneeskundige rechtspersoon ofwel er een contract mee hebben: "*Deze dierenartsen zijn vennoten van de diergeneeskundige rechtspersoon of hebben een contract met deze rechtspersoon.*" Ingaand op de casus-

(1) 19 MAART 2014. - Wet tot wijziging van de wet van 19 december 1950 tot instelling van Orde der Dierenartsen
(2) 19 DECEMBER 1950. - Wet tot instelling van de Orde der Dierenartsen.
(3) 28 AUGUSTUS 1991. - Wet op de uitoefening van de diergeneeskunde.

sen van de vraagstellers wil dat dus zeggen dat in de beide gevallen een DRP kan worden opgericht: in het eerste geval een waarbij beide dierenartsen vennoot zijn, in het andere geval een waarbij de ene dierenarts vennoot is van de DRP en de andere voor de DRP werkt op zelfstandige basis.

Aanvullend stelt Artikel 4: "*De dierenartsen en de diergeneeskundige rechtspersonen mogen het beroep van dierenarts niet uitoefenen zonder door een beroepsaansprakelijkheidsverzekering gedekt te zijn. Wat de diergeneeskundige rechtspersonen betreft, zijn alle zaakvoerders, bestuurders en leden van het directiecomité hoofdelijk aansprakelijk voor de betaling van de verzekeringspremies. De diergeneeskundige rechtspersoon kan deze verzekering in hun naam afsluiten. De diergeneeskundige rechtspersonen die het beroep uitoefenen, zijn burgerlijk aansprakelijk voor de betaling van boetes en de uitvoering van herstelmaatregelen waartoe hun organen en aangestelden veroordeeld zijn."*

Zoals reeds vermeld, moet een DRP lid zijn van de Orde (en dus lidgeld betalen), wat volkomen begrijpbaar is, gezien hij diergeneeskundige handelingen kan stellen. Daarnaast kan de DRP kiezen om een erkenning aan te vragen bij de overheid om zodoende mee te kunnen werken aan officiële overheidsopdrachten (Artikel 4): "*Daarenboven moeten de dierenartsen of diergeneeskundige rechtspersonen die meewerken aan de uitvoering van wets- en verordeningsbepalingen, vooraf erkend worden door de Minister bevoegd voor de Volksgezondheid of door zijn afgevaardigde. De Koning bepaalt de voorwaarden en de procedure voor het verlenen van de erkenning. Hij bepaalt de rechten en de plichten van de erkende dierenartsen 2 en erkende diergeneeskundige rechtspersonen]2 en regelt de wijze waarop zij vergoed worden voor het verlenen van hun diensten. Hij bepaalt de sancties die kunnen worden opgelegd bij het niet naleven van de erkenningsvoorwaarden, van de plichten en van de wets- en verordeningsbepalingen aan de uitvoering waarvan de erkende dierenartsen of erkende diergeneeskundige rechtspersonen meewerken*. Pas als een DRP erkend is door de overheid, kan hij bijvoorbeeld contracten van epidemiologische bewaking of diergeneeskundige bedrijfsbegeleiding tekenen, wat relevant is voor de vraagstellers.

Artikel 16 van de wet stelt dat: "*Alle statuten van de diergeneeskundige rechtspersonen, de overeenkomsten tussen dierenartsen, tussen een dierenarts en een diergeneeskundige rechtspersoon of tussen diergeneeskundige rechtspersonen alsook de huishoudelijke reglementen met betrekking tot die overeenkomsten worden ter goedkeuring voorgelegd aan de Gewestelijke Raad van de bevoegde Orde. De Hoge Raad van de Orde van Dierenartsen bepaalt de voorwaarden waaraan de overeenkomsten, statuten en huishoudelijke reglementen met betrekking tot die overeenkomsten dienen te beantwoorden, met name de voorwaarden in geval van ontbinding, overlijden, tuchtstraf en administratieve sanctie. De aandelenregisters en de identiteit van de mandatarissen en zaakvoerders worden meegedeeld aan de Gewestelijke Raad van de Orde.*" Voor zover ik weet heeft de Hoge Raad nooit de vermelde voorwaarden bepaald, en worden aan Waalse en Vlaamse zijde DRP-en door de respectievelijke Gewestelijke Raden goedgekeurd door zich te baseren op de wetgeving (onder andere op de hierboven vermelde bepalingen) en op de bestaande deontologische bepalingen rond de deontologische vennootschappen met diergeneeskundig doel zoals vermeld in onder andere Artikel 25 en Bijlage 4 van de Code der Plichtenleer[1] (zie verder).

"Light version" versus "full option"

Het blijft gissen of de wetgever met de invoering van de DRP enkel de bedoeling had enkele praktische problemen uit de nutsdierenpraktijk op te lossen, met name in het kader van de epidemiologische bewaking en de diergeneeskundige bedrijfsbegeleiding (waaronder het probleem dat binnen een groepspraktijk met meerdere dierenartsen slechts de dierenarts die het contract van bedrijfsbegeleiding tekende met de veehouder, geneesmiddelen mocht achterlaten binnen de tweemaandenregel, wat bijvoorbeeld lastig was tijdens vakanties of weekenddiensten; de rol van de plaatsvervanger buiten beschouwing latend), dan wel om de DRP toe te laten alle diergeneeskundige handelingen te stellen (steeds via zijn dierenartsen natuurlijke personen, vennoten of contractueel voor hem tewerkgesteld) en de dierenartsen in aanvulling op de verplichte beroepsaansprakelijkheid toe te laten hun beroepsaansprakelijkheid te beperken in navolging van de architectenvennootschap, de zogenaamde Laruelle-vennootschap. Het is namelijk binnen een DRP niet langer de dierenarts die de diergeneeskundige handelingen stelt maar de DRP zelf, via de dierenarts natuurlijke persoon. Dat is uiteraard zeer belangrijk indien er sprake is van beroepsfouten gemaakt in het kader van de uitoefening van de diergeneeskunde. Spijtig genoeg is de wetgever onvoldoende duidelijk geweest en blijft het - ik val in herhaling- gissen naar de ware bedoelingen: enkele praktische problemen oplossen, dan wel de dierenartsen net zoals de architecten toe te laten hun beroepsaansprakelijkheid te beperken. Voor de volledigheid verwijs ik daarvoor naar de bijdrage[2] van Meester Burgelman in het online beschikbaar naslagwerk "Actualia veterinair recht" [1] (editors: Luk Burgelman en Sarne De Vliegher, Larcier uitgeverij) alwaar dieper wordt ingegaan op deze boeiende materie.

Los daarvan is het vandaag dus mogelijk dat een DRP de contracten van epidemiologische bewaking (varkens[2], runderen[3] en pluimvee[4]) en van de diergeneeskundige bedrijfsbegeleiding [5] ondertekent.

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(1) 15 FEBRUARI 1995. - Koninklijk besluit houdende bijzondere maatregelen van epidemiologisch toezicht op en preventie van aangifteplichtige varkensziekten.

(2) 28 FEBRUARI 1999. - Koninklijk besluit houdende bijzondere maatregelen van epidemiologisch toezicht op en preventie van aangifteplichtige runderziekten.

(3) 17 JUNI 2013. - Koninklijk besluit tot vaststelling van veterinairrechtelijke voorschriften voor het intracommunautaire handelsverkeer en de invoer uit derde landen van pluimvee en broedeieren en tot vaststelling van de toelatingsvoorwaarden voor inrichtingen voor pluimvee.

(4) 15 FEBRUARI 1995. - Koninklijk besluit houdende bijzondere maatregelen van epidemiologisch toezicht op en preventie van aangifteplichtige varkensziekten.

(5) 28 FEBRUARI 1999. - Koninklijk besluit houdende bijzondere maatregelen van epidemiologisch toezicht op en preventie van aangifteplichtige runderziekten.

Concreet

Indien een dierenarts of enkele dierenartsen een DRP wenst/wensen op te richten dan zullen daartoe statuten moeten worden opgemaakt (of gewijzigd indien vertrokken wordt van een bestaande vennootschap) door een notaris. Deze moeten, met specifieke vermelding dat het gaat over een DRP zoals bepaald in de wet van 1950(1), in draftversie worden overgemaakt aan de bevoegde Gewestelijke Raad en zullen rekening houden met bovenstaande wettelijke en volgende deontologische bepalingen: "*Art. 25 Vennootschappen / associaties / samenwerkingen: De dierenartsen die zich wensen te associëren en/of een vennootschap wensen op te richten in het kader van de uitoefening van de diergeneeskunde moeten zich onderling verbinden met een geschreven contract, volgens de modaliteiten nader omschreven in bijlage 4. Alle geschreven contracten, alsmede eventuele akten van oprichting inhoudende de statuten en de reglementen van inwendige orde, dienen in de vorm van een ontwerp aan de Gewestelijke Raad te worden medegedeeld. Deze laatste onderzoekt of ze al dan niet in overeenstemming zijn met de diergeneeskundige plichtenleer, geeft er zijn goedkeuring aan of vordert dat passende wijzigingen worden aangebracht. Elke aanpassing of wijziging aan een eerder goedgekeurd contract dient aan dezelfde instantie voorafgaandelijk ter goedkeuring te worden medegedeeld.*"
en Bijlage 4:

"*4. Contracten tussen dierenartsen*

4.1 Elke geschreven overeenkomst moet minstens nauwkeurig opgeven:

1. het onderwerp van de overeenkomst,

2. de uitbatingszetel van de onderneming,

3. de aangewezen gesprekspartner,

4. de rechten en de plichten van de ondertekenaars,

5. de voorwaarden in geval van onbeschikbaarheid, van vertrek, van overlijden, van toetreding, van tijdelijke of definitieve uitsluiting, van ontbinding, van disciplinaire schorsing,

6. de modaliteiten van praktijkvoering en van permanentie voor zover deze georganiseerd wordt. Deze overeenkomst kan een clausule bevatten betreffende een niet-concurrentiebeding, beperkt in tijd en ruimte. Elk associatiecontract moet, buiten de hierboven vermelde verplichtingen, de modaliteiten van de verdeling van de erelonen nauwkeurig omschrijven.

4.2 In de overeenkomsten is iedere clausule verboden die:

1. de onafhankelijkheid of de beroepsverantwoordelijkheid van de dierenarts beperkt;

2. een monopoliserend karakter vertoont;

3. de vrije keuze van de cliënt beperkt;

4. een commerciële uitoefening van de diergeneeskunde met zich mee kan brengen of enige vorm van collusie.

4.3 Daarenboven moeten de vennootschappen beantwoorden aan de volgende voorwaarden:

1. de naam mag niet monopoliserend zijn;

2. alle aandelen dienen op naam te zijn;

3. de aandelen kunnen slechts toebehoren of overgedragen worden aan dierenartsen die ingeschreven zijn op de lijst van de Orde, behoudens afwijking toegestaan door de Gewestelijke Raad;

4. de bestemming van de aandelen in geval van overlijden, uitsluiting of vertrek moet gespecificeerd worden;

5. de bestuursfuncties moeten door dierenartsen worden waargenomen."

Eens de statuten en het reglement van inwendige orde zijn goedgekeurd door de bevoegde Gewestelijke Raad van de Orde, wordt de DRP opgericht via de notaris en wordt de DRP ingeschreven op de specifieke, daarvoor voorziene lijst van de Orde en krijgt hij zodoende een ordenummer (startend bij R0001). De DRP wordt daarop uitgenodigd zijn lidgeld te betalen. Op de vraag of de Orde het Federaal Agentschap voor de Veiligheid van de Voedselketen op de hoogte moet stellen van de oprichting van de DRP ben ik geneigd negatief te antwoorden gezien het Agentschap bij het tekenen van nieuwe contracten van epidemiologische bewaking en bedrijfsbegeleiding door de DRP (in de vraagstelling is er sprake van rundveepraktijken) daarvan binnen die context op de hoogte zal worden gesteld.

Daarna wordt, indien dat gewenst is in het kader van medewerking aan officieel werk voor de overheid waaronder de epidemiologische bewaking (in de beide gevallen uit de vraagstelling dus zeker van toepassing), via een louter administratieve procedure de DRP erkend door de overheid (i.e. Federale Overheidsdienst Volksgezondheid, Veiligheid van de Voedselketen en Leefmilieu). De eedaflegging zoals deze dient te gebeuren als een dierenarts natuurlijke persoon erkend wil worden, is hier om evidente redenen niet aan de orde.

De Gewestelijke Raad en de overheid worden steeds door de DRP, die daartoe een gesprekspartner aanduidt, op de hoogte gebracht van aanpassingen aan de statuten (die steeds opnieuw moeten worden goedgekeurd) en op de hoogte gehouden welke dierenartsen werken voor de DRP in kwestie.

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NAAM VAN HET DIERGENEESMIDDEL: RISPOVAL RS+PI3 Intranasal KWALITATIEVE EN KWANTITATIEVE SAMENSTELLING: Bevat per dosis van 2 ml: Werkzame bestanddelen: Gemodificeerd levend Bovine Parainfluenza type 3 (PI3V) virus, thermosensitieve stam RLB103, tussen 105,0 en 108,6 CCID50. Gemodificeerd levend Bovine Respiratory Syncytial (BRSV) virus, stam 375, tussen 105,0 en 107,2 CCID50, HULPSTOFFEN: OPLOSMIDDEL: Natrium chloride 18 mg Water voor injectie 2 ml Indicaties voor gebruik met specificatie van de doeldiersoort(en) Voor actieve immunisatie van kalveren met of zonder maternale antilichamen, vanaf een leeftijd van 9 dagen tegen BRSV en PI3V ter vermindering van de gemiddelde titer en de uitscheidingsduur van beide virussen. De aanvang van de immuniteit: 5 dagen voor BRSV en 10 dagen voor Pi3V na een enkele vaccinatie. De immuniteitsduur: 12 weken na een enkelvoudige dosis. De immuniteitsduur van de Pi3V fractie kan verminderen bij MDA positieve kalveren wanneer deze gevaccineerd worden voor de leeftijd van 3 weken. CONTRA-INDICATIES: Geen. BIJWERKINGEN (frequentie en ernst) : Uit publicaties is gebleken dat in zelden voorkomende gevallen een hypersensitiviteit-reactie opgewekt kan worden door herhaalde blootstelling aan BRSV. Dieren, waarbij het colostrum ontzegt werd, gevaccineerd aan 10 x de aanbevolen dosis voor hun derde levensweek: een toename van de temperatuur, nutritionele diarrhee, abnormale faeces and gedragingen werden vastgesteld. DOSERING & TOEDIENINGSWEG: Reconstitueer het vaccin door het oplosmiddel aseptisch toe te voegen aan de gevriesdroogde fractie. Goed schudden. Vaccinatieschema: Een enkelvoudige dosis van 2 ml gereconstitueerd vaccin intranasaal toedienen met behulp van de intranasale applicator aan rundvee vanaf een leeftijd van 9 dagen. Aanbevolen wordt om de applicator na elk dier te vervangen om het overbrengen van infectieuze organismen te vermijden.

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