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VLAAMS DIERGENEESKUNDIG TIJDSCHRIFT

2018, vol. 87, nr. 3

INHOUD

Coverfoto: Pascale Van Rooij

Rectozicht van een ***Microsporum gypseum*-isolaat** bij een paard (cf. pg. 139) op een Sabouraud-dextrose-agar na zeven dagen incubatie bij 25°C. De identificatie van dermatofyten (en schimmels in het algemeen) was lang enkel mogelijk na het opgroeien van de schimmel uit een letsel (wat niet altijd even makkelijk was) en was voornamelijk gebaseerd op de beschrijving van enkele, zowel macroscopische als microscopische, fenotypische kenmerken. Het uitvoeren en interpreteren van deze technieken vereisen voldoende kennis en kunde van en ervaring in de (veterinaire) mycologie. Tegenwoordig zijn er verschillende technieken beschikbaar die het identificatieproces van schimmels kunnen versnellen, automatiseren of objectiveren. MALDI-TOF kan bijvoorbeeld gebruikt worden om schimmels in een vroeg groeistadium op een snelle, betrouwbare en goedkope manier te identificeren, op voorwaarde dat een uitgebreide databank ter beschikking is. Methodes op basis van de detectie van DNA, zoals PCR, kunnen ook gebruikt worden zonder dat de schimmel eerst moet opgroeien, maar kunnen meestal slechts één of een beperkt aantal genera of species tegelijk detecteren.

Tekst: Filip Boyen

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Ascending placentitis in the mare

Ascenderende placentitis bij de merrie

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ABSTRACT

Ascending placentitis in the mare, which affects 3 to 7% of pregnancies, is a common cause of abortion, premature birth and delivery of compromised foals (Troedsson, 2003; LeBlanc, 2010). Since the infection ascends from the caudal genital tract, the first and most distinct lesions are seen near the caudal pole area of the allantochorion adjacent to the cervix.

The symptoms are not always obvious or will be exhibited only at a later stage of the disease process, which renders timely adequate treatment difficult. Moreover, experimental models of placentitis in the mare are difficult to maintain and double-blind, controlled studies are scarce, making it hard to formulate clear science-based advice. In this paper, the diagnosis is discussed on the basis of the symptoms, the ultrasound examinations and the endocrinological parameters, and the therapeutic and prognostic considerations are evaluated.

SAMENVATTING

Een ascenderende infectie van de placenta wordt bij 3 tot 7% van de drachtige merries gezien. Het is een veel voorkomende oorzaak van abortus, premature geboorte en zwak geboren veulens (Troedsson, 2003; LeBlanc, 2010). Gezien de infectie opklimt vanuit de caudale geslachtstractus, zijn de eerste zichtbare en meest uitgesproken letsels te vinden ter hoogte van de caudale pool van het allantochorion waar dit tegen de baarmoedermond aan ligt.

De symptomen zijn niet altijd even duidelijk of worden pas heel laat in het ziekteproces opgemerkt. Dit maakt een tijdige diagnose en initiatie van therapie uitermate moeilijk. Experimentele modellen om placentitis te bestuderen zijn moeilijk te managen en dubbel-blindexperimenten met controlegroepen zijn zeldzaam, waardoor het moeilijk is “evidence-based” advies te formuleren. In dit artikel wordt de diagnose, gebaseerd op de symptomatologie en het echografisch en endocrinologisch onderzoek, besproken en worden enkele therapeutische en prognostische aspecten benadrukt.

INTRODUCTION

Although the incidence of placentitis in pregnant mares is low, i.e. between 3 and 7%, it may lead to abortion, stillbirth or the birth of weak foals (Giles et al., 1993; Hong et al., 1993a; Barr, 2005) (Figure 1). Besides the costs of extra neonatal care, which is often necessary when a viable foal is born, stillbirth and abortion lead to loss of income and the affected mare may be subfertile in the subsequent breeding season. Of all abortions, premature deliveries and perinatal deaths in horses, about 10 to 30% can be attributed to placentitis. As such, placentitis is a common cause of perinatal mortality (Giles et al., 1993; Hong et al., 1993b; Smith et al., 2003; Troedsson, 2003; Laugier et al., 2011; Lyle, 2014).

Pathologically, three different forms of placentitis are described, namely ascending, diffuse and focal placentitis (Zent et al., 1999). Ascending placentitis, which is caused by bacteria ascending through the vagina and infecting the placenta, is the most prevalent form of placentitis (Platt, 1975a; Whitwell, 1988; Hong et al., 1993a; Zent et al., 1999).

Placentitis is mostly of bacterial origin, but in

Figure 1. Placenta lesions in a placentitis case. Placental lesions and mucoid discharge at the level of the cervical star with a thickened allantochorion in a mare with placentitis and premature delivery of a compromised foal.

about 15% of cases, a fungal or mixed infection is seen (Smith et al., 2003). Most commonly, opportunistic bacteria from the lower reproductive tract of the mare are involved, with mainly *Streptococcus equi subsp* zooepidemicus isolated, in addition to *Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* (Platt, 1975b; Merkt, 1985; Hinrichs et al., 1988; Whitwell, 1988; Acland, 1993; Lyle, 2014).

In the majority of cases, the affected mares are pluriparous and may have anatomic defects of the genital tract, such as pneumo- or urovagina and/or cervical incompetence (Platt, 1975b; Macpherson and Bailey, 2008; LeBlanc, 2010; Löf et al., 2014). No breed predisposition has been noted. However, due to breeding regulations and policies, Thoroughbreds might be overrepresented (Macpherson and Bailey, 2008a).

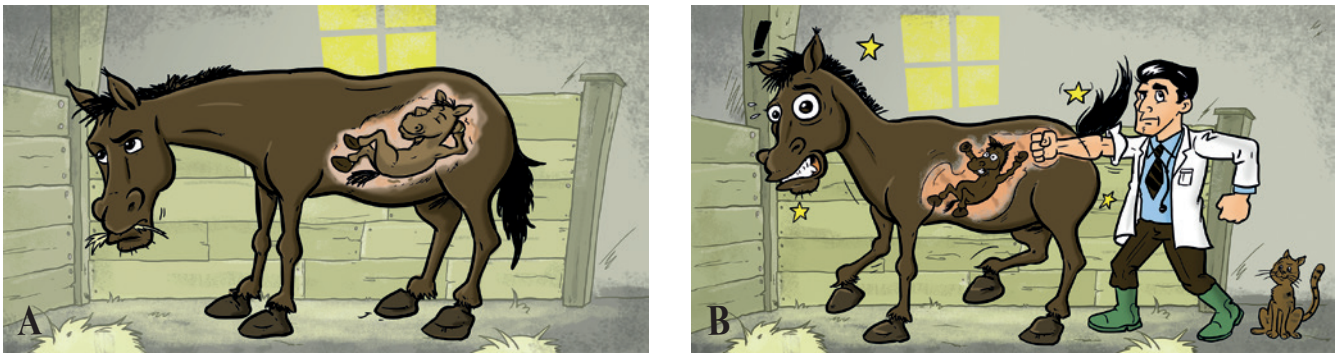
The infection, which ascends from the caudal genital tract and is facilitated by poor perineal conformation and/or cervical incompetence, is initiated at the cervical pole (Whitwell, 1988; Mays et al., 2002). In most cases, ascending placentitis occurs in the last trimester of gestation, when traction of the enlarged uterus may aggravate conformational abnormalities and the malfunctioning of the pelvic diaphragm, cervix and perineum. As a result of the infection, the fetal membranes become edematous and thickened, and they might separate from their attachment to the uterus (Platt, 1975b). The infection or the inflammation of the placental membranes alone causes a decreased functionality of the membranes, the release of pro-inflammatory cytokines, such as TNFα, IL-1β, IL-6 and IL-8 from placental and fetal tissues (Dudley, 1997; LeBlanc et al., 2002; Mays et al., 2002) and, together with cortisol from the mare, activation of the fetal hypothalamo-pituitary-adrenal-axis (HPA) and an increase in fetal cortisol (Challis et al., 2000). This increase in cortisol decreases 15-hydroxy prostaglandin dehydrogenase (PGDH), which in turn leads to increasing prostaglandine (E2 and F2alpha) concentrations (LeBlanc et al., 2002; McGlothlin et al., 2004). The pro-inflammatory cytokines also increase the cyclo-oxygenase -2 (COX-2) activity (Lyle, 2014), which in turn increases the synthesis of prostaglandins. As a result of the decrease in prostaglandin dehydrogenase (PGDH) and the premature increase of COX-2, PGE2 and PGF2alpha, myometrial contractions and premature labor occur (Keelan et al., 1997; Romero et al., 1998; Gravett et al., 2000; Gibb and Challis, 2002). Thus, it is the inflammation, which is a consequence of the infection, that induces premature abortion or preterm delivery of the fetus rather than the bacterial infection per se (Pollard and Mitchell, 1996, Leblanc et al., 2002; Mays et al., 2002; McGlothlin et al., 2004; Lyle, 2009). The above described pathway, however, is a simplification of the complex mechanisms leading to abortion; for a more detailed description of the pathogenesis of placentitis, the authors refer to Lyle (2014).

There is a scarcity of evidence-based medicine to support the development of therapeutic approaches, since not only are experimental infection trials expensive and difficult to maintain, most mares also abort without any warning symptoms, since placentitis in a clinical setting externalizes itself as an insidious, hidden disease for which the diagnosis often comes too late (Macpherson and Bailey, 2008a). Moreover, endocrine regulation in the late-term mare differs from that in other species, with only subtle serum changes occurring very late in gestation, just before expulsion.

SYMPTOMS

Early diagnosis enables rapid intervention and improves the prognosis and pregnancy outcome. Unfortunately, in most cases, the diagnosis is difficult to make since the mares are rarely ill, and they do not have abnormal blood counts or changes in clinical parameters. The earliest sign, vaginal discharge, is not seen in every case (Renaudin et al., 1999; Morris et al., 2007; Macpherson and Bailey, 2008a) and the subsequent premature development of the udder is not a constant sign (Zent et al., 1999; LeBlanc et al., 2002; Morris et al. 2007; Bailey et al., 2010; LeBlanc, 2010). Although in some cases, no symptoms at all can be seen externally, even in cases, where premature udder development is seen without vaginal discharge, the mare must be checked for the presence of twins, for nocardioform (focal) placentitis and for ascending placentitis (LeBlanc, 2010).

Vaginal exploration, whether manually or through a speculum, is contraindicated. The vestibule of the mare harbors a variety of germs that may be pushed to the cranial part of the vagina during such an examination (Hinrichs et al., 1988; Bucca and Fogarthy, 2011) (Figures 2A and 2B). Moreover, an accidental mechanical stimulation of the cervix during such an



Figures 2A and B. A vaginal exploration is contraindicated. The vestibule harbors a variety of germs that may be pushed to the cranial part of the vagina. Moreover, stimulation of the cervix during such an examination can induce premature parturition.

examination can induce premature parturition (Handler et al., 2003). The tone, size and patency of the cervix can be assessed in a non-invasive way by rectal palpation and ultrasonography. The degree of cervical relaxation can be helpful for estimating the prognosis of the affected gestation. However, in mares, from the tenth month on, cervical scores (assessing length, width, echoic characteristic and tone) approach 'prepartum scores' and are, as such, only of limited use in late gestation (Bucca and Fogarthy, 2011).

ULTRASONOGRAPHY

The combined thickness of uterus and placenta (CTUP) can be measured by transrectal ultrasonography using a linear probe (5-7.5MHz) at the ventral portion of the placenta just cranially to the cervix. This is easier to perform when using the vasculature of the uterine branch of the vaginal artery, with its course ventral to the uterine border a little bit to the lateral side (on both sides), as a landmark (Renaudin et al., 1997; Macpherson 2006; Bucca and Fogarthy, 2011) (Figures 3A and 3B). As such, one measures always at the same location, and the hypoechoic area of the blood vessel makes it easier to visualize the contours of the uteroplacental delineation. In a normal gestation, the placenta cannot be distinguished from the uterine wall (Macpherson, 2006), although this also depends on the time of gestation (more obvious at the end of term) and the resolution of the ultrasound equipment used.

The CTUP thickens physiologically with increasing gestational age (Renaudin et al., 1997; Kelleman et al., 2002; Morris et al., 2007). A pathologically thickened CTUP can be seen in an inflamed placenta and the separation of the allantochorion from the uterine wall may become visible. Sometimes, fluid and pus can be seen between the chorion and the uterus in more advanced cases of placentitis (LeBlanc et al., 2004). In short, a CTUP of more than 1.2 cm at eleven months of gestation, or more than 1.5 cm at twelve months of gestation, may be associated with placentitis (Renaudin et al., 1997; Troedsson et al., 1997; Troedsson, 2001; Bucca et al., 2005, Bucca 2006). The thickening can develop quite rapidly, depending on the degree of contamination and inflammation, and in infectious trials, it has been observed within 7 to 48 hours post inoculation (Renaudin et al., 1999; Bailey et al., 2012).

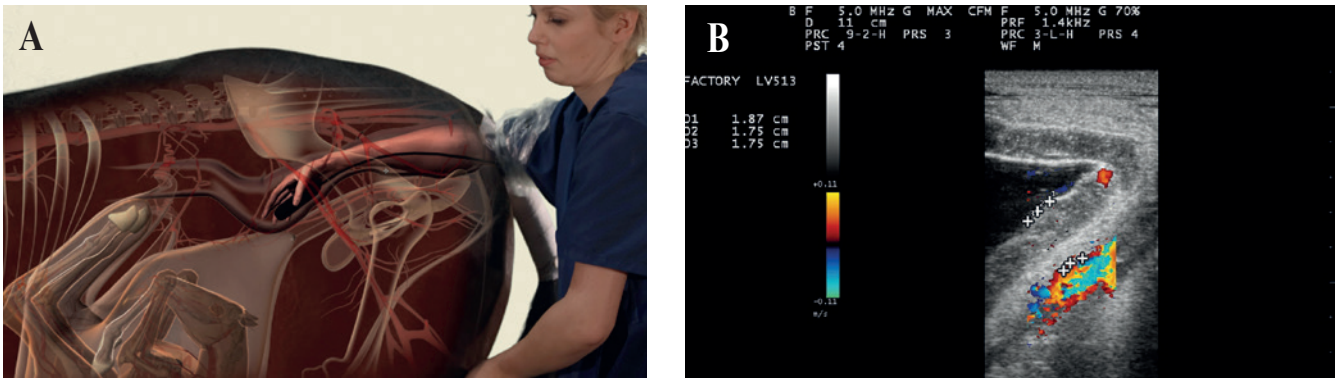


Figure 3 A. The measurement is taken just cranial of the cervix at the level of the arterial communication between a branch of the a. vaginalis and a. uterine media as a landmark. B. Transrectal ultrasonographic image of the combined thickness of uterus and placenta (CTUP) in a placentitis case. Transrectal ultrasonographic image of the CTUP (in between +-+). Increased thickness (depending on gestational age), the presence of edema and/or placental separation from the uterine wall can be indications of placentitis.

In some studies (Morris et al., 2007; Bailey et al., 2010; Löf et al., 2014), no different CTUP measures between mares with or without placentitis were seen, which led the authors to the conclusion that CTUP is not a good indicator of ascending placentitis during the final month of gestation in Thoroughbred mares (Löf et al., 2014). However, if an increase in CTUP is seen, a degree of placental insufficiency may be present (Cummins et al., 2008), but also other reasons of thickened placenta should be considered (e.g. fescue toxicosis, twin gestation) (Hudson et al. 2005; Govaere et al., 2009; Souza et al. 2010). Moreover, Souza et al. (2010) concluded that CTUP measurement in mares should not be the only parameter used to estimate placental failure and impending abortion.

Bailey et al. (2012) evaluated the use of Doppler measurements to diagnose placentitis in a clinical setting. However, since the onset of the disease occurs when the uterine blood flow is already very high, no differences can be detected between affected and healthy cases; therefore this parameter should not be used.

Not only the aspect and size of the CTUP have to be checked, also the appearance of the fetal fluids should be evaluated. Cloudy allantoic fluids in the last three months of gestation may indicate infection (LeBlanc, 2010), although in a study by Renaudin et al. (1999), no echoic changes were noted, neither in the amniotic nor in the allantoic fluid of the affected mares. While the fetal fluids are being checked, the fetal viability parameters (e.g. movement and heart rate) should also be assessed (LeBlanc, 2010). Repeated ultrasound measurements and check-ups are necessary to minimize the risk of error, since abrupt fetal movements can also cause the whirling up of cellular material, which produces a high density of vernix without clinical implications (Macpherson and Bailey, 2008a).

The well-being of the fetus and the extent of the inflammation should be evaluated not only on the basis of the symptoms in the mare, but also on the basis of the transrectal and transabdominal ultrasound examinations of the placenta, fetal fluids and fetus, and of the serum hormone profiles (LeBlanc, 2010).

ENDOCRINE PROFILES ASSOCIATED WITH PLACENTITIS

In the last trimester of pregnancy, the feto-placental functionality plays an important role in the endocrine regulation of pregnancy and parturition. Endocrine profiles in the late-term pregnant and periparturient mare are the result of a delicate interplay of changing hormone levels and interactions that must coincide all in a timely and concerted manner to ensure a smooth birth of a viable foal. Any condition that affects the functionality of the fetus or the placenta also alters the endocrine production, thus possibly disrupting this interaction. The feto-placental functionality can be monitored by measuring progestins and/or estrogens (Ousey, 2006; Morris et al., 2007). However, while the disease process can alter the endocrine pathways and stimulate inflammatory and immune responses, these alterations are only usable for diagnosis after the fact (Rossdale et al., 1991; Santschi et al., 1991; Ousey et al., 2004, 2006; LeBlanc, 2010).

When looking at progestin levels, it is advised to evaluate at least three consecutive samples obtained within a two- to three-day interval (Morris et al., 2007). Different abnormalities in progestin patterns have been described (LeBlanc, 2010). In short, a premature rapid decline is observed in acute conditions with imminent fetal expulsion or in the event of a dead fetus (Ousey, 2006). An early rise in progestin serum concentrations is associated with placental pathology or fetal stress (Rossdale et al., 1992; Ousey et al., 2006; Morris et al., 2007). When these elevated progestin concentrations are maintained for two to three weeks prior to the 310th day of gestation, then a certain degree of fetal HPA activation can be assumed. These foals are more likely to survive than those that have not had this period of activation (Le Blanc, 2010). Another abnormality in the progestin pattern is the failure to exhibit the normal prepartum rise in progestin level, as is seen almost exclusively in mares after ergopeptine alkaloid exposure (fescue toxicosis) (Brendemuehl et al., 1995).

In the mare, maternal progestin concentrations are low until three weeks before parturition, when they start to rise with a subsequent abrupt decrease 24 hours before parturition (Ousey et al., 2003). This rise is associated with the onset of mammary electrolyte secretion while the fall in concentration is simultaneous with the rise in fetal cortisol levels (LeBlanc, 2010). These alterations in electrolyte concentrations in mammary secretions can be used to predict fetal readiness in the normal, a term mare. However, in preterm mares, it can be an indicator of impending abortion (Ousey et al., 1984; Rossdale et al., 1991).

In most commercial assays (RID and ELISA), progestin cross-reacts with progesterone and as such can be used to assess progestin concentration in the mare. However, the cross-reactivity of a test may differ, resulting in different progestin concentrations measured. Of the three tested commercial laboratories in Belgium, only one lab was able to detect the total progestin concentrations alongside the progesterone concentration (Figure 4). Therefore, it is essential to know the concentration in normal pregnant mares of the assay used, in order to discriminate concentration changes in placentitis cases (Ousey, 2006).

Progestin values of between 2 and 12ng/ml are found up until the last three weeks in uncompromised gestations (Ousey et al., 2005; Morris et al., 2007), and consecutive samples vary less than 15% within a 24-hour period (Morris et al. 2007). Decreased progestin levels are indicative of compromised foals,

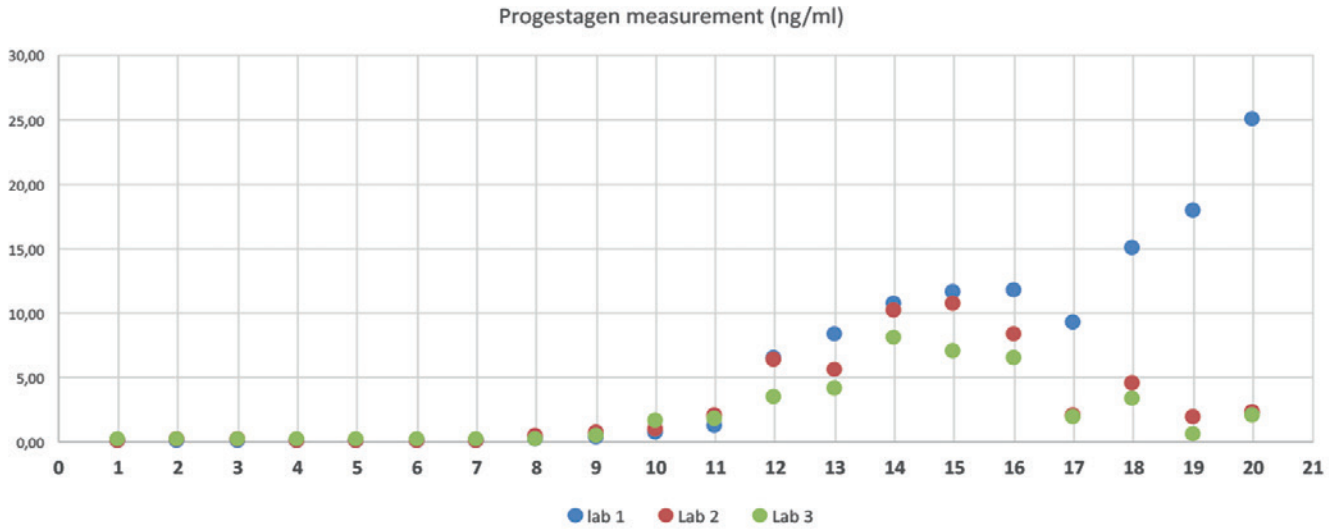


Figure 4. Differences in serum progestogen measurements in the second half of gestation, depending on laboratory. Serum progestagen measurements in 21 mare samples performed in three different commercial laboratories in Flanders. Mares 1-16 are normal cycling mares; mares 17-21 are in the second half of an uncompromised pregnancy. A clear discrepancy can be noted between the results. Only lab 1 seems to be able to determine other progestagens than progesterone in the second half of pregnancy in the mare.

though elevated levels beyond day 305 of gestation cannot be differentiated from the normal physiological rise in progestin levels in the last three weeks of uncompromised gestations (Morris et al., 2007).

In a clinical setting, mares can be monitored by sampling three times a week, or by taking four samples at 48-hour intervals in the acute phase and biweekly thereafter to assess the effectiveness of therapy (Morris et al., 2007; Macpherson and Bailey, 2008a). When treatment is successful, the CTUP measurements diminish rapidly, and the progestin levels and clinical symptoms tend to normalize.

Besides progestins, estradiol is sometimes used as a marker for feto-placental compromise. Estrogen precursors (C19) are secreted by the fetal gonads. In contrast to what is seen in the fetal adrenal glands, fetal gonads do not respond to stress and as a result, the estrogen levels only change in severely compromised, almost ill-fated pregnancies (Ousey, 2006). Concentrations above 1000ng/ml between 150 days and 280 days are assumed to be normal; while concentrations less than 500ng/ml have been associated with severely compromised or dead foals (LeBlanc, 2010).

Relaxin, a polypeptide that is mainly produced by the placenta during pregnancy (Stewart et al., 1982; Klonisch et al., 1995; 2000), has been shown to promote uterine growth (Hall et al., 1990; Hall et al., 1992), inhibit myometrial contractions (Watts et al., 1988) and loosen the tissues of the caudal genital tract in preparation for parturition in many species (Grahm and Darcy, 1952; Steinitz et al., 1959; Kertiles and Anderson, 1979; MacLennan et al., 1980; O'Day et al., 1989; Ryan et al., 1999). Alterations in relaxin blood profiles are seen in women with impending miscarriage (Witt et al., 1990; Stewart et al., 1993) and in dogs suffering early embryonic loss (Gunzel-Apel et al., 2008).

In mares, relaxin serum levels are high (varying from 45.0-85.0ng/ml) at end of gestation and increase during labor. Plasma relaxin declines markedly in mares with problematic pregnancies (Ryan et al. 1999). Previous work, however, has shown that relaxin concentrations can vary and are different between breeds and are thus of limited value as a placentitis marker in the mare (Klonisch et al., 1995; Ryan et al., 2009). Nevertheless, the one (and only) proven use of plasma relaxin in mares is its use as a non-invasive marker of placental function, to be considered in addition to and as a complement to other diagnostic findings (Ryan et al., 1999).

Prostaglandins are released locally by the uteroplacental tissues and are rapidly metabolized. Therefore, they cannot be used for diagnosis of placentitis (LeBlanc, 2010).

Other serological markers for diagnosing placetitis are far from specific. Acute phase proteins (haptoglobulin and serum amyloid A (SAA)) will rise as a response to the infection, with SAA showing a marked increase. The finding that SAA increases in response to placentitis gives new insights into the disease process. This increased concentration of SAA can only be explained in terms of the contribution of the endometrium to this rise or, although always regarded as a local process, in terms of its systemic involvement in the pathogenesis of placentitis (Canisso et al., 2014). The fibrinogen levels and the white blood cell count do not change post inoculation (Canisso et al., 2014). Other serum markers, such as alpha-fetoprotein (AFP), are still under research (Canisso et al., 2015).

TREATMENT

Treatment should aim at reducing the spread of the germs, eliminating the inflammation, reducing cytokine signalling, preventing prostaglandin synthesis and thus preventing uterine contractions and preterm delivery of the fetus (LeBlanc, 2010; Lyle, 2014). To obtain these targets, a combination of antibiotics and anti-inflammatory drugs, whether or not supplemented with hormones, are most currently given as therapy.

Parturition induction in order to obtain better chances for the foal's survival is not an option (Jeffcott and Rossdale, 1977; Rossdale and Silver, 1982; Leadon et al., 1986). The foal and its chances of survival benefit from any delay of preterm parturition. When a premature expulsion can be avoided, the maturation of the foal in a chronic stress situation is accelerated. This increases its chances of survival by a significant degree (Rossdale et al., 1991; LeBlanc et al., 2004; Ousey, 2006; Bailey et al., 2007; Christiansen et al., 2009). The artificial acceleration of foal maturation with the aid of cortisone (e.g. dexamethasone I.M., at a dose rate of 100mg q 24 hours for three consecutive days) is sometimes considered, although it has variable effects, depending on the product used and the degree of HPA activity (Alm et al., 1975; Jeffcott and Rossdale, 1977; Christiansen et al., 2005), and it is not without risks due to the side effects of the corticosteroids (Rossdale et al., 1992; Ousey et al., 2006).

In addition to their effects on the fetal HPA, corticosteroids also have an immunosuppressive effect that can affect prostaglandin production (Gravett et al., 2000). Even so, PG synthetase inhibitors (e.g. meclofenamic acid) (Silver et al., 1979) and cyclooxygenase inhibitors (e.g. flunixin meglumine, at a dose rate of 1.1 mg/kg b.i.d.) reduce prostaglandin 2 alpha concentrations, without inhibiting the oxytocin induced delivery a term (Ousey, 2006).

The most commonly used antimicrobial to treat placentitis is trimethoprim sulfamethoxazole (TPS), which has good oral bioavailability in horses (Zent, 1999), although its activity may be reduced in the presence of pus, and some strains of Streptococci are resistant to it (Peyrou et al., 2003). Therapies with a combination of TPS and pentoxifylline (Pf) prolong gestation but do not prevent stillbirth (Graczyk et al., 2006). Penicillin and gentamycin have also been used, though their concentrations in the allantoic fluid reach only about 80% of the serum concentrations, whereas TPS and Pf reach serum concentrations in the allantoic fluids (Murchie et al., 2006; Rebello et al., 2006). In affected mares, the gentamycin concentrations are reduced below the minimal inhibitory concentrations (MIC) of gramm-negative bacteria, whilst the clearance of penicillin G from the allantoic cavity is delayed, resulting in concentrations that are possibly toxic for the fetus (Murchie et al., 2006). Specific posology and dosages of therapeutics in cases of placentitis are reviewed in LeBlanc et al. (2010) (Table 1).

The administration of pentoxifylline (Pf) is based on its anti-inflammatory properties by reducing the amount of cytokines (TNFα and IL-1) (Lauterbach et al., 1996; Baskett et al., 1997, Ousey et al., 2010). Although previously presumed effective, even a double dose of Pf (a ratio of 17mg/kg BID) does not alter the arterial blood flow (Bailey et al., 2012). In earlier tri-

Table 1. Dosage and posology of therapeutics (adapted from: LeBlanc, 2010). The AMCRA recommendations. Ease of use and cost price, which depend on the pharmaceutical formulation and vary from country to country, and as such most appropriate for the situation in Belgium. The withdrawal times and legal implications of administration, which may differ between countries and change over time, should be consulted up front.

		AMCRA code	Withdrawal (FP)	Dose	Posology	Application	Ease of use	Cost price
Antimicrobial	Trimethoprim sulpha	1	14 d	15-30 mg/kg	q 12h	p.o.	*	*
	Potassium penicillin G		6 mth	22000 IU/kg	q 6h	i.v.	***	**
	Gentamycin	2	6 mth	6.6 mg/kg	q 24h	i.v.	**	**
	Ceftiofur	3	6 mth	20 mg/kg	q 12h	i.v./i.m.	**	***
Tocolytic	Altrenogest		9 d	0.088 mg/kg	q 24h	p.o.	*	*
	Isoxsuprine			0.4-0.6 mg/kg	q 24h	p.o.	*	
Anti-inflammatory	Flunixin meglumine		7-10 d	1.1 mg/kg	q 12-24h	i.v./p.o.	*	**
	Phenylbutazone		NFP	2.2 mg/kg	q 12-24h	p.o.	*	*
	Pentoxifylline		6 mth + pass	8.5 mg/kg	q 12h	p.o.	*	*
	Acetylsalicyl zuur			50 mg/kg	q 12h	p.o.	*	*
Anti-inflammatory + stimulation fetal maturation	Dexamethasone		8 d	40-35-25 mg (decrease dose every 48h)	q 24h	i.v.	*	*

*: low cost / easy to use , ***: high cost / more difficult to use

FP = food producing/ Non-FP

als, when given for a prolonged period of time, Pf did increase uterine blood flow, thus ameliorating oxygen transport (Bacher et al., 1997; 2005). By altering the flow characteristics of equine erythrocytes in vitro (Weis et al., 1994), Pf might help in bacterial clearance, thus impeding colonization, as shown in rabbits (Heller et al., 1999). Normal therapies use a dosage of 8-10 mg/kg q 12 hours, with maximal concentrations being reached in about one hour post administration (Liska et al., 2006). For similar empirical reasons, acetylsalicylacid (150mg/kg, p.o., b.i.d.) is sometimes advocated.

Besides antimicrobials and anti-inflammatory medication, several drugs that act to impede any preterm contraction of the uterus (β-sympathomimetics, prostaglandin synthesis inhibitors, calcium channel blockers, oxytocin antagonists) have been tested in humans (Lamont 2005), though none of them were able to prolong the pregnancy or improve the prognosis when used alone. In mares, the anti-prostaglandin effect of progesterone (analogues) prevents prostaglandin induced abortion in most cases in the first trimester of gestation (Daels et al., 1996). Apparently, the upregulation of oxytocin and prostaglandin receptors is inhibited at that stage by the administration of progesterone, and without these receptors and the formation of gap junctions between the myometrial cells, uterine contraction cannot happen (Garfield, 1980). The specific action and efficacy of progesterone supplementation as a way to prevent abortion in late gestation still remain equivocal (Ousey, 2006). Since the plasma progesterone concentrations are increased in most of these placentitis mares, supplementation is questionable. Some authors believe that it might be contraindicated, since a supplement of P4 inhibits placental 3β-HSD, converting endogen P5 to P4 (Chavatte et al., 1995; Schutzer et al. 1996). A total blockage of 3β-HSD leads to parturition in most animals, but not in the mare (Fowden et al. 1987; Silver and Fowden,1988; Chavatte et al. 1997). Most likely, other progestogens play a more determining role in preventing preterm birth in the mare (Ousey, 2006). Nevertheless, to date, progesterone supplementation remains a part of the standard therapy protocol in placentitis cases. Commonly, a dosage of altrenogest of 0.088mg/kg bwt q 24 hour per os is used.

When β-sympathomimetics such as clenbuterol are used in the mare, uterine relaxation is seen within minutes, and lasts for up to two hours (Card and Wood, 1995). However, no significant difference in gestation length has been seen (Palmer et al., 2002). Moreover, treated mares tend to foal earlier than controls, as clenbuterol may induce cervical relaxation and subsequent parturition; hence, clenbuterol has only limited use in placentitis in mares (Palmer et al., 2002).

The duration of placentitis therapy (antibiotics, NSAID's, altrenogest) is still under debate. Cures of 10-14 days of antibiotics with seven days of NSAID's have been advised by some, while others advise to treat until delivery of the foal (Zent et al., 1999; Le Blanc, 2010). Prolonged antibiotic and NSAID therapy may result in gastrointestinal side effects and antibiotic resistance (LeBlanc, 2009). Protracted progestin therapy up to the time of delivery may lead to prolonged stage 2 and subsequent neonatal complications (Neuhauser et al., 2008). It is therefore advisable to stop progesterone therapy at 320 days of gestation (LeBlanc, 2010). To complicate the discussion even more, in most placentitis mares, even after a prolonged antibiotic therapy, uterine swabs post foaling still show bacterial growth (Bailey et al., 2010).

Early and aggressive combination therapy is necessary until clinical signs disappear (Rebello et al. 2006, Christiansen et al., 2009; LeBlanc, 2009), associated with regularly checking the effect of the therapy by monitoring the viability of the foal (Macpherson and Bailey, 2008a; LeBlanc, 2010).

Mares that suffer from placentitis should be placed under close surveillance to enable assisted delivery of the foal, since the thickened placenta prevent in most cases a timely rupture of the cervical star leading to a so called 'red bag' delivery. After foaling or abortion, they should be checked for endometrial infection for the purpose of selecting the most appropriate therapy. When this check-up for infection is delayed, the samples will always reveal a mixed infection (Macpherson and Bailey, 2008b; Bailey et al., 2010). In most cases, three consecutive days of uterine flushes in combination with NSAID's and a week of antimicrobial therapy clear the infected post-partum uterus.

Logically, after placental expulsion, a thorough examination of the placenta is essential. Bacterial sampling will not be of any use, but a visual check-up for gross lesions (especially at the caudal chorionic part and cervical star region) and histological examination of the region of interest can help to diagnose placentitis (Mays et al., 2002). In the event of ascending placentitis, a thickened, discolored, edematous or even ulcerated chorioallantois is present at the level of the cervical star (Platt, 1975b; Hong et al., 1993; Mays et al., 2002) (Figure 1). Gross lesions at macroscopic inspection are not specific, as they may be absent in the event of placentitis or be caused by other diseases, as in cases of cervical pool necrosis (Löf et al., 2014). Furthermore, the ultrasonographic feature of a thickened placenta is not correlated with the total weight of the placenta after expulsion (Löf et al., 2014); however, a heavier placenta may be caused by inflammation and should be sampled for histology to exclude placentitis.

PROGNOSIS

As mentioned above, the mare's health status is not affected unless severe complications occur. The prognosis for the fetus is acceptable when early, aggressive therapy can be initiated; however, in a first-

line approach, this is quite difficult due to the insidious course and discrete signs of placentitis (Bailey et al., 2010).

In any case, longer intervals from parturition to subsequent pregnancy (with on average one month extra) have been noted in affected Thoroughbred mares compared to normal Thoroughbred mares (Hughes et al., 2014).

Foals born from affected mares should be checked meticulously for signs of pre- or dysmaturity and septicemia. The description of the whole scale of controls and therapeutic considerations relating to the neonate is beyond the scope of this paper. In any case, measurement of the foal serum cortisol level in addition to the blood analysis, with specific attention to the neutrophil/lymphocyte ratio, should be part of the first screening. The foals should be supervised closely until there is certainty – and even more so in the presence of suspected signs (clinical signs during pregnancy and/or placental gross lesions). In addition to strictly monitoring the colostrum intake and the essential viability parameters, antibiotic therapy should also be started unless it is deemed unnecessary.

Foals born out of chronically infected and inflamed placentas, in association with high sero-progestin levels in the dam, have some (premature) degree of fetal HPA activity and increased cortisol levels, which promote their survival (Ousey, 2006). However, elevated levels of glucocorticoids, together with the limited nutrient and blood supply due to the reduced placental bloodflow, cause a reduction in fetal body weight and may be associated with poor skeletal development and limb deformities of the neonate (Ousey, 2006). Surviving foals may need expensive intensive care and may possibly never reach the expected levels of athletic performance. In the US, the costs of clinical care for such foals born out of such compromised gestations have been estimated to run between $2,000 and over $10,000 (Barr, 2005). This is a consideration that should be communicated to the owner before initiating such therapy (LeBlanc, 2010). In any case, pre- and postnatal epigenetic conditions determine the possible athletic capacities of the offspring in its later life (Axon et al., 1999; Hughes et al., 2014).

An examination of the Timeform ratings of three-year-old race horses has revealed that only very few of those born with low birth weight reached standard ratings, regardless of their origin (Platt, 1975b; 1978). The fact that Hughes et al. (2014) could not see any differences in their retrospective study looking at the performance of yearlings and two-year-olds born out of compromised pregnancies versus controls born out of uneventful pregnancies, must be viewed in light of the fact that the pre-test criteria included mainly subclinical cases of placentitis and (of course) the fact that only the surviving foals were taken into account.

Finally, in addition to mares that show clinical signs of ascending placentitis, also mares that have suffered from placentitis in a previous pregnancy should be monitored from the seventh month of gestation onwards. Since the pathogenesis usually involves an aberrant functionality of the physical barriers guarding the sterility in utero, when this dysfunctionality remains unresolved (or is unresolvable), the infection-inflammation may emerge in any of the subsequent pregnancies.

CONCLUSION

Recognizing an ascending infection of the placenta in the pregnant mare can be difficult since the symptoms may be discrete, and/or they may be exhibited only in a late stage of the disease. Nevertheless, the process of elucidating the entire pathological pathway may result in the development of other diagnostic and therapeutic tools. So far, the evaluation of the progestogen profiles of suspected mares, in association with transrectal ultrasound evaluation of the placenta, seems to enable the diagnosis of most placentitis cases adequately (Morris et al., 2007). However, initiating early and aggressive combination treatment (antibiotics, anti-inflammatory drugs and altrenogest) can be difficult and, as a result, the prognosis remains guarded. Foals born out of a compromised gestation should be systematically and rigorously monitored for pre- or dysmaturity and septicemia. Affected mares should be examined in order to eliminate any underlying causes. Any mare at risk needs to be examined frequently during the subsequent gestation, from the seventh month onward, to ensure timely diagnosis should placentitis reoccur.

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Paratuberculosis in dairy and pygmy goats: an underestimated problem?

Paratuberculose bij melk- en dwerggeiten: een onderschat probleem?

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ABSTRACT

Paratuberculosis is a chronic intestinal disease affecting goats and other ruminants worldwide. The objective of this review was to summarize current knowledge on the prevalence, diagnostic possibilities and possible prevention and control measures for paratuberculosis in dairy and pygmy goats in Europe. The herd level prevalence of *Mycobacterium avium subspecies paratuberculosis* (MAP) in dairy goat farms is very high, namely 71%, 63% and 86% in Germany, France and the Netherlands, respectively. The prevalence in pygmy goats is undocumented. Antibody ELISA, fecal culture and PCR testing are readily available for diagnosis. A highly suggestive finding for paratuberculosis is the presence of enlarged intestinal lymph nodes on abdominal ultrasonography. To control the disease a combination of 'test and cull' and environmental hygienic measures are recommended. In contrast to cattle, vaccination is available and might be an additional tool in the control program for goats. Vaccine antibodies are no longer detectable after one year, and therefore serological monitoring can be continued on vaccinating herds. The worrisome prevalence data from neighboring countries indicate the need for a prevalence study on dairy goat farms in Belgium.

SAMENVATTING

Paratuberculose is een chronische, intestinale aandoening bij geiten en andere herkauwers. Het doel van dit overzichtsartikel is om de huidige kennis omtrent prevalentie, diagnostische mogelijkheden, preventie en mogelijke controlemaatregelen tegen paratuberculose bij melk- en dwerggeiten in Europa samen te vatten. De bedrijfsprevalentie van *Mycobacterium avium subspecies paratuberculosis* (MAP) is in de ons omringende landen zorgwekkend hoog, namelijk 71%, 63% en 86% in Duitsland, Frankrijk en Nederland, respectievelijk. De prevalentie bij dwerggeiten is onbekend. Antistof ELISA, cultuur en PCR van mest zijn vlot beschikbaar om de diagnose te bevestigen. Een sterk suggestief letsel voor paratuberculose is de aanwezigheid van vergrote intestinale lymfeknopen op abdominale echografie. Om deze aandoening onder controle te krijgen, is een combinatie van "test and cull" en hygiënische maatregelen sterk aangeraden. In tegenstelling tot bij rundvee is vaccineren mogelijk en zou het een bijdrage kunnen leveren tot controleprogramma's voor paratuberculose bij geiten. Vaccinantistoffen zijn niet meer detecteerbaar één jaar na vaccinatie, waardoor serologische monitoring mogelijk blijft op gevaccineerde bedrijven. De verontrustende prevalentiecijfers in aangrenzende landen tonen de dringende noodzaak van een prevalentiestudie op melkgeitenbedrijven in België aan.

INTRODUCTION

Paratuberculosis is a chronic contagious disease caused by *Mycobacterium avium subspecies paratuberculosis* (MAP) (Verdugo et al., 2014), affecting several animal species: predominantly ruminants (cattle, sheep, goat, deer, etc.), but also camelids, rabbits, foxes, pigs, horses, llamas, alpacas, deer and weasels are

susceptible (Windsor, 2015). Animals get infected at a very young age, but due to the long incubation time, clinical signs appear much later; on average at the age of two (Mercier et al., 2010).

Paratuberculosis has been less studied in goats than in cattle (Nielsen et al., 2008). There are important differences between both species regarding their susceptibility and clinical presentations of paratuberculosis. Goats have a less efficient humoral immunity reaction to MAP (Kostoulas et al., 2006) and clinical illness develops at an earlier stage in the pathogenesis. In contrast to cattle, chronic diarrhea is not a prominent clinical sign in goats (Robbe-Austerman et al., 2011), and MAP bacteria are almost never found in goat's colostrum (0%) or milk (4%), even when antibodies against MAP are detected (Lievaart-Peterson, 2017). Another important difference with cattle in the control of paratuberculosis is that vaccines against MAP are available for goats and sheep. These inactivated vaccines induce antibodies, which might interfere with the use of serology as a diagnostic tool in control programs (Sevilla et al., 2008).

The aim of this review is to provide an overview of the current knowledge on the prevalence of paratuberculosis in goats, the different diagnostic possibilities and difficulties, possible prevention and control strategies and the influence of vaccination on prevalence studies and control programs.

MATERIALS AND METHODS

A search was conducted in different databases (PubMed, Web Of Science and Google Scholar) with the following key words: goat, paratuberculosis, diagnosis, prevalence, MAP, prevention and vaccination.

PREVALENCE OF PARATUBERCULOSIS IN EUROPE

In Europe, peer-reviewed prevalence studies have been published in Germany, France and the Netherlands. In Germany, 1609 unvaccinated animals (1473 sheep and 136 goats) (10 animals with the lowest body condition score in each farm) were tested using serum enzyme linked immunosorbent assay (ELISA) (sensitivity (SE) 90% and specificity (SP) 99%, as determined in cows. Of the sheep and goats, 14% and 21% tested positive, respectively. The herd-level prevalence on 167 farms, i.e. 150 sheep and 17 goat farms, was 65% and 71%, respectively. The within-herd prevalence was 21% and 32%, in sheep and goats, respectively (Stau et al., 2012). In France, in 2010, 105 dairy goat farms were screened with an antibody ELISA (SE= 53% and SP= 100%), covering 11,847 unvaccinated goats older than six months old. Of the farms, 62.9% (95% confidence interval (CI)= 41.4 – 84.4) tested positive and the within-herd prevalence was 5.5% (95% CI= 2.62 – 3.24) (Mercier et al., 2010). In the Netherlands, 36 dairy goat farms were tested using antibody ELISA; 86% (95% CI= 72% - 94%) of the farms tested positive. In contrast to France and Germany, 26 farms of this Dutch population vaccinated their goats against paratuberculosis. All vaccinating farms tested positive (95% CI= 87% - 100%). Of the ten non-vaccinating herds, only 50% (95% CI= 19% - 81%) tested positive. Depending on the cut-off, the within-herd prevalence varied between 4% (0% - 7%) and 8% (0% - 16%) (Lievaert-Peterson, 2013). It is important to bear in mind that these studies report apparent prevalence, and given the low SE of some ELISAs, true prevalence might be different. For Belgium, no peer-reviewed prevalence studies are available. In an unpublished study, nine commercial dairy goat farms of the approximately 60 specialized dairy goat farms in Belgium were tested by sampling 10% of the animals aged between two and five years old. All tested positive and the within-herd prevalence ranged between 11% and 39% (Vicca, unpublished results). The only identified herd-level risk factor for paratuberculosis is increasing herd size (Stau et al., 2012). This suggests that the prevalence of paratuberculosis in the European countries studied is much higher in goats than in cattle (3-27% true prevalence and 7-55% apparent prevalence on antibody ELISA) (Garcia and Shalloo, 2015). Data on the prevalence in pygmy goats are currently lacking, but case reports are available (Tuerlinckx, 2017). The close contact between pygmy goats and humans and the fact that goat's cheese has been more frequently consumed in recent years, warrant a closer estimate of the possible zoonotic risks (Statistics Belgium, 2016).

Figure 1. Marked weight loss in a dairy goat with paratuberculosis (front, marked with pink).

DIAGNOSTIC POSSIBILITIES AND DIFFICULTIES

The incubation time of paratuberculosis is long and highly variable (Robbe-Austerman, 2011). In goats, the main clinical signs are cachexia, production loss, a dull and crustaceous hair coat, exercise intolerance (Boelaert et al., 2000; Windsor, 2016), selective food intake (leaving the concentrate) and sometimes intermittent diarrhea (Maynard-James et al., 1997). In a study on 54 goats with paratuberculosis, 54% had normal feces, 24% had pasty feces, 15% had intermittent diarrhea and only 7% suffered from chronic diarrhea. Dairy goats generally don't reach the 'diarrhea stage', possibly because they are removed earlier due to declining production. In contrast, pygmy goats do get to the diarrhea stage (GD, 2014). The only systematic clinical sign is cachexia without anorexia (Fernandez-Silva et al., 2014) (Figures 1 and 2). Therefore, it is impossible to diagnose paratuberculosis only by clinical observation and examination (Bastida et al., 2011).

Several diagnostic tests are available: on the one hand culture, polymerase chain reaction (PCR) analysis and Ziehl-Neelsen staining of feces or intestinal or lymphoid tissue, and on the other hand, detection of antibodies in serum or milk by ELISA. The long and variable incubation time (one to four months at 37°C) (Eriks et al., 1996) makes isolation of MAP from feces difficult. Fecal culture has a low SE (8% (2% - 17%)) because of the difficult in vitro incubation, the intermittent shedding of MAP and the unequal division of bacteria in the feces (Kostoulas et al., 2006). Ziehl-Neelsen solution stains mycol-acid in the bacterial membrane. *Mycobacteria* are able to hold the reddish color despite decoloring attempts with alcohol. Therefore, *Mycobacteria* will turn red on a blue background. The Ziehl-Neelsen procedure is not specific because all *Mycobacterium spp.* color red (Navarro et al., 1991). Moreover, the SE is low (36.4% in cows) (Zimmer et al., 1999).

Next to culture and Ziehl-Neelsen staining, antibody ELISA can be performed on milk or serum. The SE and SP for milk and serum are comparable, and ELISA on milk has been suggested as a good screening tool for dairy goat farms (Windsor, 2015). Since 2007, the Flemish Animal Health Service (Diergezondheidszorg Vlaanderen) offers an antibody ELISA on milk with a SE of 86.7% and a SP of 99.7%, in Belgium. The problem with ELISA is that antibodies are detected, which are only present during the humoral T_H2-immunity stage, after the cell-mediated T_H1-immunity stage. The T_H2-immunity is suppressed by the cell-mediated T_H1-immunity, which starts shortly after infection. Therefore, there is a high risk of false negative results when testing serologically with ELISA before the humoral T_H2-immunity stage (Kostoulas, 2005). ELISA is not suitable to quantify the number of MAP bacteria and to detect paratuberculosis before the humoral stage. The most recently available test is PCR, which has the highest SE (94.1%) and SP (100.0%) to detect an infection. The test can be applied on feces, intestinal and lymphoid tissue or milk. It allows to differentiate low and high shedders. This method has only recently been implemented in the dairy goat industry, since the PCR-test has become cheaper and more accurate (Bastida et al., 2011).

Figure 2. Detail of the lumbar region in a dairy goat with cachexia as a consequence of paratuberculosis.

The gold standard for diagnosing clinical paratuberculosis in goats remains post-mortem examination (Robbe-Austerman, 2011). The following pathological findings can be seen: depletion of fat depots, muscle atrophy, thickening of the intestinal wall and enlargement of the mesenteric lymph nodes (Windsor, 2015). Mesenteric lymph nodes enlarge up to 60 – 70 mm x 20 – 30 mm. Several white-yellow spots of necrosis may be visible on the cutting surface. Sometimes goats suffering from clinical paratuberculosis show mineralized nodules in the cortex of the lymph node (Lybeck et al., 2015). To confirm the diagnosis of paratuberculosis at necropsy, histology with hematoxylin-eosin staining can be used. In some cases, a chronic granulomatous enterocolitis with infiltration of macrophages, lymphocytes and plasma cells in the lamina propria can be noticed (Fernandez-Silva et al., 2014).

In goats, there are roughly two kinds of histological findings, namely diffuse and local lesions (Windsor, 2015). Diffuse lesions show a granulomatous infiltration of the mesenteric lymph nodes and the intestinal wall, with transmural enteritis. The intestinal villi are shortened, enlarged and erosion and ulceration of the intestinal wall are sometimes remarked. In contrast, in goats with local lesions, multiple granulomas and a high number of lymphocytes are present in the intestinal wall and mesenteric lymph nodes. The intestinal wall is not as enlarged as it is in goats with diffuse lesions, and often the epithelia of the intestine are unaffected (Lybeck et al., 2013). Further detail on the histopathological findings is available elsewhere (Windsor, 2015). A final diagnosis can only be made by testing feces or tissue samples by PCR or culture (Robbe-Austerman, 2011). Furthermore, Ziehl-

Neelsen staining can be applied on ileal or mesenteric lymph node samples.

An alternative method to detect goats infected with paratuberculosis is ultrasonography. This method has become more accessible with pygmy goats being kept as pets. Their owners are more willing to pay for a more individual and specialized diagnostic approach. With a 7.5 MHz transducer penetrating up to 12 centimeters, a standing goat can be examined. Shaving is often needed as only making contact with alcohol and/or gel on the goat coat often provides insufficient image quality. Three aspects of the small intestine need to be visualized to suspect paratuberculosis: (1) the thickness and the (2) presence of folds in the mucosa of the intestinal wall and the size of mesenteric lymph nodes. Tharwat (2012) found that only 44% of 54 seropositive goats had a thickened intestinal wall (2 mm up to > 5.1 mm), the other 56% showed no thickening of the intestinal wall (< 2mm). Transversal folding of the mucosa of the intestinal wall was not a very reliable criterion, since only 13% of the seropositive goats showed this on ultrasound. The most sensitive ultrasonographic finding was the enlargement of the mesenteric lymph nodes. Of goats with paratuberculosis, 91% (n=49) showed this image on echography (confirmed by necropsy) (Figure 3). Of these goats, 73% showed a hypo-echogenic cortex and hyper-echogenic medulla. None of the mesenteric lymph nodes of the control population could be visualized by ultrasound (Tharwat et al., 2012).

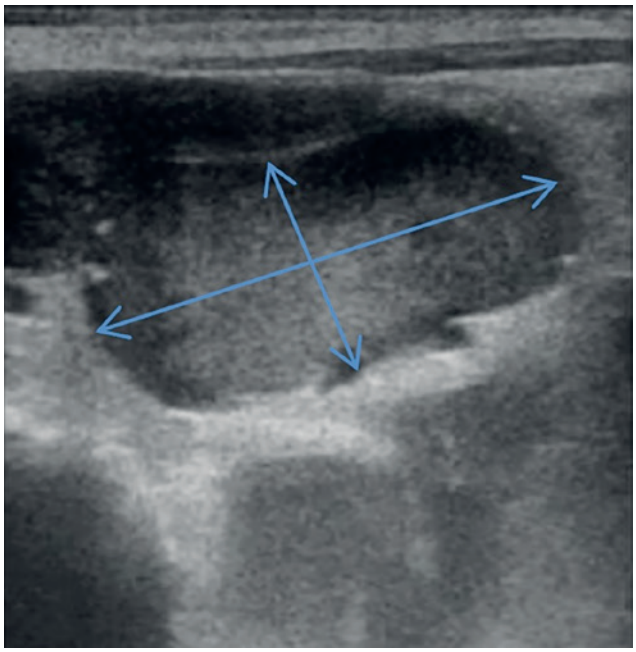

Figure 3. Ultrasonographic appearance (7.5 MHz probe) of a typical enlarged mesenteric lymph node (3.85 cm x 1.71 cm) in a 2.5-year-old pygmy goat suffering from paratuberculosis.

PREVENTION AND CONTROL

To the authors' knowledge, in Europe, paratuberculosis programs in goats are only available in the Netherlands (voluntary) and in Norway (obligatory for herds delivering to certain dairy companies); this is in contrast to the situation in cattle. Most cattle paratuberculosis programs are based on the principles of 'test and cull' and decreasing pathogen transmission by improved management (hygiene and in extenso translated into external and internal biosecurity) (Bastida et al., 2011). The same two principles can be applied to goats, but also vaccination can be applied in replacement stock to increase their resistance to infection (Windsor, 2015). The oro-fecal route is the most common route of infection in goats. An infected goat sheds up to 10^8 MAP per gram of feces (Windsor, 2015). Also colostrum and milk are considered infectious; however, in contrast to cattle, colostrum apparently plays a minor role in goats (Leonor Mundo et al., 2013). In a recent Dutch survey, MAP could not be detected in any of 121 colostrum samples, whereas 95% contained MAP antibodies (Lievaart-Peterson, 2017). In addition, transplacental infection is possible in goats (Manning et al., 2003). MAP survives for over one year in the environment and can be retrieved from airborne dust particles. Not only the farm itself but also neighboring fields where cows, sheep or other goats are grazing are a reservoir of MAP (Robbe-Austerman, 2011). Moreover, wildlife might contaminate pasture or drinking ponds; however, up till now, only wild ruminants (deer, etc.) and lagomorphs have been evidenced to shed MAP in their feces of fecal shedding, thereby spreading MAP by contaminating the environment (Stevenson et al., 2009).

To prevent farms from being infected, purchased animals need to be tested. However, given the low sensitivity, only relying on a purchase test is insufficient. In addition, it is strongly advised to only purchase animals from a herd of origin with a known negative paratuberculosis status. Also male goats should be tested (Robbe-Austerman, 2011). Given the currently estimated high prevalence in Belgium, purchase from negative herds will be difficult.

In positive herds, control measures need to be implemented to gradually reduce the prevalence and eventually become negative. Three main pillars to control paratuberculosis in goats have been mentioned: management measures to decrease pathogen spread, 'test and cull' and vaccination. The most important management measure to avoid spread is motherless raising. Goat milk is a potential source (in 4% of the cases (Lievaart-Peterson, 2017)) of MAP. Separation of the lambs immediately after birth and subsequent prolonged individual housing are still recommended (Robbe-Austerman, 2011). Even though the presence of MAP in colostrum has not been demonstrated in Dutch dairy goats (Lievaart-Peterson, 2017), replacement colostrum from other MAP free goat or cattle

farms is recommended. Alternatively, colostrum of positive farms might be pasteurized. Low-temperature long-time pasteurization (60°C during 60 minutes) is the only possibility, because colostrum antibodies are destroyed at higher temperatures. In a small-scale study, in each of four replicate batches, no viable MAP could be retrieved after such a pasteurization protocol (Godden et al., 2006). However, there is no consensus on the 100% efficacy of this procedure. Moreover, pasteurization of small amounts of colostrum is practically difficult: most devices work with 1-4 liter bags. It should be noted that in cattle, this pasteurization protocol has been shown to be ineffective in the long term, most likely because animals become infected through the environment later in life (Godden et al., 2015). In contrast to cattle, the effects of supplemental hygienic and combined external and internal biosecurity measures on the incidence of new MAP infections in goat herds have not been studied yet.

The traditionally most important pillar of a paratuberculosis control program is a test-and-cull policy. In the dairy goat industry, test-and-cull is controversial, since dairy goats have a high economic value. Another issue is the large number of false negatives due to the low sensitivity of the different diagnostic tests (Windsor, 2015). In the Netherlands, a voluntary paratuberculosis control program has recently been initiated for dairy goats. The program is based on serial (annual) antibody ELISAs on milk or serum and differentiates three statuses: status A= no infection, status B= infected animals and animals with antibodies are culled; status C= infected animals and animals with antibodies are not culled. A difference with the Dutch paratuberculosis program for cattle is that no fecal culture confirmation is required for goats. Unfortunately, to date, only 5% of the Dutch dairy goat farms participates (personal communication, M. Holzhauer, GD, the Netherlands). The program does not include vaccination. In Norway, all goat farmers delivering milk to the dairy industry are obliged to check for antibodies against MAP on bulk tank milk five times a year. In Australia, a national, voluntary Johne's Disease Control Program is in action and depends on the widespread use of vaccination (Windsor, 2015).

THE SIGNIFICANCE OF VACCINATION FOR MONITORING AND CONTROL PROGRAMS

An important difference with cattle is that a goat vaccine against MAP is available for control programs (Gudair®, CZ Veterinaria, Porriño, Spain). This vaccine with heat inactivated MAP bacteria is registered in Spain and the Netherlands. The vaccination scheme includes a once-in-a-lifetime vaccination at the age of 2-3 weeks up to 6 months of all animals (including adults at initiation of the herd vaccination). A meta-analysis has shown that vaccination reduces microbial contamination, and reduces or delays production losses and pathology (Batista and Juste, 2011). However, vaccination can not fully prevent infection and large variations between the available vaccines have been shown. In Australia, the national paratuberculosis control program uses the Gudair® vaccine. Since the introduction of this vaccine in 2002, in Australia it is believed to be the most economical way of controlling paratuberculosis (Windsor, 2015). The results are twenty times more satisfying than the test-and-cull strategy using serum ELISA (Bastida et al., 2011). An average decline of damage and infection of 94.8% (measured in effect at tissue level), 79.3% of the epidemiology (microbiological infection risks, measured in number of isolations of MAP in feces or tissue) and 45.1% less production losses (losses measured as number of clinical cases or deaths) have been shown in the Netherlands (GD, 2014). A study of 1998 revealed that, when vaccinating with the Gudair® vaccine, mortality and the prevalence of MAP shedders declined with 90%, and shedding started twelve months later (Dhand et al., 2016). However, shedding could not be prevented. Different studies have shown very good results; however, the results were not as satisfying in populations with a low or medium prevalence. Therefore, the high expectations of the Gudair® vaccine can only be met when vaccinating a population with a high prevalence, as currently in Belgium (Windsor, 2013). Vaccination should be continued, even after the whole population is immunized. Shedding of MAP may continue up to six years, meaning that even buying vaccinated goats poses a risk of introducing MAP (Windsor, 2013). Remarkably, many of the available vaccination studies do not include a control group (Bastida et al., 2011). In conclusion, based on the available data, the effects of vaccination should not be overestimated; moreover, it appears to be impossible for a farm to become negative only by vaccination (Windsor et al., 2014). Nevertheless, in Australia, the control program with vaccination has been of enormous economic benefit (Windsor, 2015).

An important disadvantage of paratuberculosis vaccination is the cross reactions with the intradermal tests for *Mycobacterium tuberculosis*, hampering national tuberculosis control programs (Windsor, 2015). Because the Gudair® vaccine is not a marker-vaccine, interference with the serological diagnosis and monitoring of goat farms might also occur. A series of Dutch studies have shown that antibody levels start to reduce 16 weeks after vaccination and can no longer be detected one year after vaccination (Lievaert-Peterson, 2013). Therefore, monitoring of vaccinated herds remains possible, but needs to be done in a representative number of animals to avoid false positives. Finally, attention should be paid to avoid self-injection when administering the vaccine as extensive local necrosis can be effected (Windsor, 2015).

CONCLUSION

Available studies show a worrisome high prevalence of paratuberculosis in dairy goats in the neighboring countries of Belgium. The prevalence in companion goats is currently undocumented. Based on this information, a prevalence study on Belgian dairy goat farms is urgently warranted. Identical diagnostic tools as in cattle are available and identical issues with the long incubation period exist. Enlargement of the mesenteric lymph nodes is a highly suggestive finding on abdominal ultrasonography. Prevention and control programs should include a combination of test-and-cull and management measures to reduce pathogen spread. In contrast to cattle, vaccination offers perspective as an additional control tool. As vaccine antibodies can only be detected until one year after vaccination, serological monitoring in vaccinated herds remains possible.

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Uit het verleden

Miniatuur (via Wikipedia commons) uit 'Livre de Chasse' (1387-1389) van Gaston III, graaf van Foix (Béarn) bijgenaamd Phoebus (de schitterende), opgedragen aan hertog Filips de Stoute. Jagen was zijn passie naast, zoals de graaf zelf verklaarde, le combat et l'amour. Meerdere exemplaren van dit werk bleven bewaard, onder andere in de Brusselse Koninklijke Bibliotheek. We zien hierin meerdere vormen van de verzorging van gekwetste jachthonden.

Luc Devriese

Successful management of a solitary simple renal cyst in a dog

Succesvolle behandeling van een solitaire simpele renale cyste bij een hond

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ABSTRACT

In this report, a dog is described that was referred to the Small Animal Department, Faculty of Veterinary Medicine (Ghent University) for further work-up and treatment of a solitary renal cyst. The cyst was treated successfully with ultrasound-guided drainage and percutaneous sclerotherapy. The dog was followed-up during twenty-four months post procedure with renal sonography. The complaints of intermittent lethargy disappeared after the first treatment. The ultrasonographic appearance of the cyst evolved from a round, anechoic, sharply demarcated lesion to an ill-defined, hyperechoic lesion.

SAMENVATTING

In deze casuïstiek wordt een hond beschreven die doorgestuurd werd naar de vakgroep Kleine Huisdieren van de Faculteit Diergeneeskunde (UGent) voor de behandeling en opvolging van een solitaire renale cyste. De cyste werd succesvol behandeld met echografisch begeleide drainage en percutane sclerotherapie. De initiële klachten van intermitterende lethargie verdwenen na de eerste behandeling. De hond werd gedurende vierentwintig maanden verder opgevolgd met renale echografie. Het uitzicht op echografie evolueerde van een ronde, anechogene, goed afgelijnde laesie tot een slecht afgelijnde, hyperechogene laesie.

INTRODUCTION

Renal cysts are epithelium-lined cavities filled with liquid of various composition (Akinci et al., 2005). Renal cysts can be classified as solitary or multiple, inherited or acquired and simple or complicated. The simple cysts do not contain cells, bacteria or fungi (Zatelli et al., 2007; Nyland et al., 2015). Agut et al. (2008) define simple cysts as cysts not associated with decreased renal function.

In human medicine, simple renal cysts are solitary, unilateral and cortical in 75% of the cases (Waterman, 2014). Pathophysiology is not well-described (Hanna and Dahniya, 1996; Zatelli et al., 2007) and the pathological significance is unclear (Zatelli et al., 2005). Simple renal cysts are mostly clinically silent in humans (Akinci et al., 2005), but they can also be associated with abdominal pain, urinary tract obstruction and systemic hypertension (Akinci et al., 2005; Zatelli et al., 2005). In human medicine, progression to neoplasia has been described (Naoky et al., 2000).

In veterinary medicine, complaints are abdominal pain, reduced physical activity, anorexia and systemic hypertension, the last being more commonly seen in cats (Zatelli et al., 2007; Agut et al., 2008). Simple renal cysts are typically incidentally detected during an abdominal ultrasonographic examination (Zatelli et al., 2005; Nyland et al., 2015).

In veterinary medicine, ultrasonography is the technique of choice for the diagnosis of cystic lesions (Paskalev, 2012). Simple renal cysts have specific ultrasonographic features of a benign lesion consisting of a well-defined cavity with anechoic content, strong distal acoustic enhancement and sharp demarcation (Nyland et al., 2015). Other differentials for a well-defined cavity with anechoic content, strong distal

acoustic enhancement and sharp demarcation should be considered. Polycystic kidney disease (PKD), seen in Persian cats and Cairn and Bull terriers, cysts seen in familial nephropathy of Shih tzus and Lhasa apsos, hereditary multifocal renal cystadenoma or cystadenocarcinoma, usually seen in German Shepherd dogs, hematoma and abscesses should be on the differential list. The last two usually contain sedimentation and can thus be differentiated from a true cyst by the presence of an echoic content (d'Anjou, 2008; Dennis et al., 2008).

In humans, treatment is necessary when clinical symptoms or urinary tract obstruction are associated with the cyst (Chung et al., 2000; Akinci et al., 2005). Different treatment options have been described but the most frequently and efficiently used method is ultrasound-guided percutaneous sclerotherapy both in human and veterinary medicine (Akinci et al., 2005, Zatelli et al., 2005, Agut et al., 2008). Sclerotherapy is a therapy where a sclerosing solution is injected in a vessel or tissue.

The complication rate of percutaneous sclerotherapy in human medicine is 1.7% (Gelczer et al., 1998), and the most common complication is abdominal bleeding (Lang et al., 1977). Other potential complications both in human and veterinary medicine include rupture or bleeding of the cyst during placement of the needle or leakage of the sclerosing solution, often alcohol, outside of the cyst (Gelczer et al., 1998; Zatelli et al., 2005; Agut et al., 2008).

CASE DESCRIPTION

History and physical examination

A ten-year-old, female spayed American Staffordshire terrier was referred to the Small Animal Department of the Faculty of Veterinary Medicine (Ghent University, Belgium) for intermittent lethargy and abdominal discomfort and further evaluation of a progressively growing renal cyst located at the caudal pole of the left kidney detected by the referring veterinarian during an abdominal ultrasonographic examination. Three months prior to referral, complete blood- and urine analysis were performed (Table 1). Repeated ultrasonographic examinations performed by the referring veterinarian showed gradual enlargement of the cyst (Table 2). Renal diet was initiated. At the time of referral, the patient was asymptomatic and no abnormalities were noted on physical examination.

Further work-up

Complete blood count and serum biochemistry profile including electrolytes did not reveal significant abnormalities. Measurement of serum symmetric dimethylarginine (SDMA), a new renal biomarker, showed a mildly increased value (Table 1). Complete urinalysis, urinary protein: creatinine ratio (UPC) and bacterial urine culture were performed on urine collected by cystocenthesis and revealed no abnormalities (Table 1). Systolic blood pressure, measured with a Doppler technique, according to the American College of Veterinary Internal medicine (ACVIM) consensus statement (Brown et al., 2007) was 140 mmHg. Ultrasonographic examination of the abdomen confirmed the presence of a large, ovoid, well-delineated, thin-walled, anechoic lesion within the caudal pole of the cortex of the left kidney (3.5 cm x 2.7 cm) (Figure 1A). There was a thin hyperechoic line (septum) extending from one side to the other in this cystic structure. The right kidney did not show abnormalities. On the basis of these findings, the diagnosis of a solitary renal cyst was suspected.

Treatment and follow-up

Percutaneous drainage and alcoholization of the cyst were performed under ultrasound guidance. Before the procedure, coagulation profile (prothrombin time and activated partial thromboplastin time) was

Table 1. Blood and urine parameters initially and during follow-up. Evolution of serum creatinine, serum symmetric dimethylarginine (SDMA), urine specific gravity (USG) and urinary protein:creatinine ratio (UPC) during several months. T0 being the day of referral to the Faculty, T-3 months the first consultation at the referring veterinarian and T 24 months the last available follow-up at the referring veterinarian.

	T -3m	T -2m	T 0m	T 1m	T 2m	T 5m	T 8m	T 24m
SDMA-value (0-14 µg/dL)	/	/	17	11	/	/	/	12
Creatinine (Reference: 44-159 µmol/L)	133.5	/	149	144	/	/	/	137
USG (Reference: 1.015-1.045)	1.031	1.012	1.024	1.023	/	/	1.008	/
UPC (Reference: <0.50)	0.06	/	0.05	0.10	/	/	/	/

Table 2. Ultrasonographic follow-up of the cyst. For legend see Table 1.

	T -3m	T -2m	T 0d	T 1m	T 2m	T 5m	T 8m	T 24m
Size cyst in cm	3 cm	3.2 cm	3.5 cm x 2.7 cm	2.41 cm x 2.31 cm	1.8 cm	0.99 cm x 0.62 cm	No cyst detectable: ill-defined hyperechoic lesion	No cyst detectable: ill-defined hyperechoic lesion
Size after alcoholization (A) /drainage (D)	/	/	1.1 cm x 0.8 cm (D+A)	No cyst detectable (D)	/	/	/	

checked and was within normal limits. The dog was premedicated with dexmedetomidine (3 µg/kg, Dexdomitor®) and butorphanol (0.2 mg/kg, Dolorex®). Induction and maintenance of anesthesia were obtained with alfaxalone (2 mg/kg, Alfaxan®). Aseptic preparation of the skin was performed before drainage. The cyst was drained under ultrasound-guided percutaneous approach using a spinal needle (22 gauge) attached to a three-way stopcock with extension set and a syringe. Thirteen milliliters of transparent fluid were successfully aspirated from the renal cyst cavity without complications. Six milliliters of sterile ethanol 95% were then injected into the cyst and re-aspirated after three minutes according to the protocol described by Zatellli et al. (2005). The quantity of ethanol injected, equivalent to one half of the drained liquid, reduces the risk of rupture or bleeding and permits good distension of the cystic wall (Zatelli et al., 2005). After aspiration, a small cavity remained at the level of the cystic area (1.1 cm x 8 mm) (Figure 1B). The aspirated fluid was clear, acellular and low in protein and bacterial culture was negative; hence, the cyst was classified as a simple renal cyst.

At one month follow-up, the dog was asymptomatic and serum creatinine, SDMA, urinalysis and systolic blood pressure (135 mmHg) were re-evaluated (Table 1). Control abdominal ultrasonography revealed a rounded fluid-filled cavity of 2.4 cm x 2.3 cm at the caudal pole of the left kidney. The cavitary fluid was slightly more echoic than the initial echogenicity of the cyst and contained a few echoic speckles.

Under general anesthesia with the same protocol as with the first drainage and alcoholization, the left renal fluid-filled cavity was drained a second time as described above and three milliliters of hemorrhagic fluid were aspirated. Alcoholization was not repeated. Cytological examination revealed the presence of a large amount of red blood cells and activated macrophages with phagocytized red blood cells. No bacteria or inflammatory cells were seen at cytological examination and bacterial culture was negative. These findings were consistent with hemorrhage into the cystic cavity. After aspiration, no fluid filled cavity was detectable. At two months post alcoholization, the cyst showed a diameter of 1.8 cm (Table 2). No medical treatment was instaured. Monthly check-ups including abdominal ultrasonography and urinalysis were advised (Table 2). Follow-up renal ultrasonography by the referring veterinarian at eight and twenty-four months after alcoholization did only reveal an ill-defined, hyperechoic lesion and the absence of cystic lesion.

DISCUSSION

Simple renal cysts are rare in veterinary medicine and pathophysiology has not yet been described. No data exist on the prevalence of renal cysts in veterinary medicine (Zatelli et al., 2002). The overall prevalence in humans is 10.7% of which 17.4% is in the fifth or later decades of life (Chang et al., 2007). In another prevalence survey, 1.700 individuals (11.9% of the total) had at least one renal cyst on sonography. The incidence increases with age and the ratio men:women is 2:1 (Terada et al., 2002; Chang et al., 2007).

Similarly to the majority of human renal cysts (Waterman, 2014), the case reported here consisted of a solitary, unilateral and cortical renal cyst. Simple renal cysts have specific ultrasonographic features. Round or oval lesions, sharply demarcated with an anechoic content, a thin hyperechoic wall and strong distal acoustic enhancement are observed (Nyland et al., 2015). Ultrasonographic appearance of the renal cyst in the patient presented in this report was compatible with all these features.

In the case described here, the content of the cyst was acellular, and bacterial culture was negative; hence, the diagnosis of simple cyst was made, according to the definition proposed by Zatelli et al (2007). On the other hand, according to Agut et al. (2008), simple cysts are not associated with decreased renal function. Despite a creatinine value within reference interval, the renal marker SDMA was increased at initial presentation suggesting a decreased renal function. The glomerular filtration rate (GFR) was not evaluated to assess the renal function more closely.

Direct measurement of GFR is the gold standard for quantitative assessment of kidney function. Due to the need of multiple urine- or blood samples, it is not routinely performed in dogs and cats (Paepe and Daminet, 2013; Pressler, 2013; Relford et al., 2016).

SDMA is a new sensitive and specific renal biomarker. It is a byproduct of protein degradation created by methylation of arginine. This substance is primarily eliminated by renal excretion (>90%) and therefore is a promising renal endogenous marker (Hall et al., 2016). SDMA strongly correlates with GFR, increases before serum creatinine in progressive kidney disease in dogs and is not influenced by muscle mass (Nabity et al., 2015; Hall et al., 2016). SDMA has been shown to increase above reference interval when about 30% of renal function is lost. SDMA has been added preliminary to the International Renal Interest Society guidelines in addition to serum creatinine in staging of early and advanced chronic kidney disease (IRIS guidelines; Relford et al., 2016). Treatment with alcoholization of the renal cyst has led to a decrease of SDMA to a value within reference interval, which shows that sclerotherapy of the cyst improves kidney function. This has not been detected by serum creatinine measurement.

In a retrospective study of Zatelli et al. (2007), ten animals were diagnosed with a renal cyst, and impairment of kidney function was not identified based on normal serum creatinine. Creatinine is the most widely used endogenous biochemical marker of GFR but has many limitations, e.g. it increases only when approximately 75% of nephron mass is lost and is influenced by muscle mass (Nabity et al., 2015; Hokamp and Nabity, 2016; Relford et al., 2016). In a study of Zatelli et al. (2007), SDMA assay was not available in dogs at that time and GFR was not performed. Therefore, a subtle decrease of renal function undetectable by serum creatinine, could have been missed.

Different therapeutic methods of a simple renal cyst have been described, drainage and sclerotherapy being the mostly used safe and effective procedure in human medicine (Akinci et al., 2004). Before performing sclerotherapy, the clinician must be sure of the diagnosis of a simple renal cyst. Performing sclerotherapy on a cystic structure with a different nature and origin may have serious consequences. Several studies have been performed to evaluate the effectiveness of single versus multiple sclerotherapy sessions for the treatment of simple renal cysts in humans (Hanna and Dahniya, 1996; Chung et al., 2000; Akinci et al., 2004). Single drainage recurrence rate has been reported between 33 and 80% (Hanna and Dahniya, 1996) and thought to be due to continuing fluid production by the epithelial cells lining the cyst (Hanna and Dahniya, 1996; Waterman, 2014). Many authors promote multiple sessions compared to single sessions to decrease recurrence rates (Hanna and Dahniya, 1996; Chung et al., 2000). In a study by Hanna and Dahniya (1996), a success rate of 68% and 100% was obtained with single sclerotherapy session and multiple sessions, respectively. Chung et al. (2000) reported similar success rates, 57% and 95%, respectively for single versus multiple sclerotherapy sessions. Akinci et al. (2004) reported an average cyst volume reduction of 93% at the end of the first year and a complete resolution of the cyst in 17.5% of the cases in patients with one single session of sclerotherapy. The authors of the present case performed only one alcoholization in the patient. As reported in the literature, the authors anticipated to perform two alcoholizations of the cyst. However, the second drainage of the cyst revealed hemorrhagic fluid and a septic lesion could not be excluded. Therefore, a second alcoholization was not performed. Since the nature of the liquid was unknown, it was opted not to perform a second alcoholization but only an aspiration to further investigate the fluid.

However, based on the size reduction of the cyst

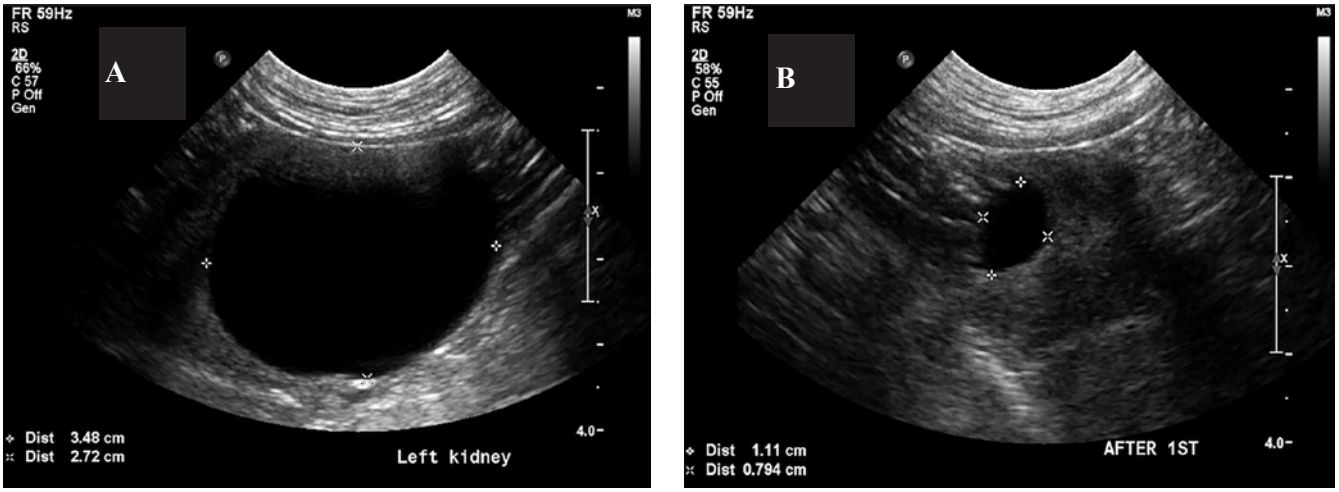

Figure 1A. Transverse ultrasonographic images of the left kidney. A large ovoid, well-delineated, thin-walled, anechoic lesion is visible within its caudal pole (3.5 cm x 2.7 cm) the day of referral to the Faculty. B. Post procedure image. Remaining of a 1.1 cm x 8 mm cystic structure at the caudal pole of the left kidney.

and decrease in serum SDMA measurement, cyst drainage and alcoholization were considered successful. Also twenty-four months post alcoholization, during ultrasonographic examination at the referring veterinarian, only an ill-defined, hyperechoic lesion was detected. The hyperechoic lesion is most likely compatible with scar tissue.

In conclusion, further studies are needed to evaluate the success and recurrence rate of single versus multiple sclerotherapy of renal cysts in veterinary medicine. In this patient, a single sclerotherapy provided complete resolution of the renal cyst and improvement of the renal function. Increased SDMA at initial presentation was compatible with mild decreased renal function that was not detected by serum creatinine.

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Microsporum gypseum infection in a horse with severe sweet itch

Microsporum gypseum-*infectie bij een paard met erge zomereczeem*

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ABSTRACT

In a horse with severe sweet itch and a history of corticosteroid treatment for this disorder, a single, well-circumscribed and crusty white lesion was present on the thigh. The geophilic dermatophyte species *M. gypseum* was cultured from the lesion. The lesion was treated topically once a week with an enilconazole emulsion for four weeks. Full recovery was noted after two months. This case suggests that sweet itch combined with systemic corticosteroid treatment, may act as predisposing factor for dermatophyte infection. In addition, the importance of correct isolation and identification of the causal agent is highlighted.

SAMENVATTING

Bij een paard met erge zomereczeem en een voorgeschiedenis van corticosteroïdebehandeling voor deze aandoening, werd een welomschreven, cirkelvormig letsel met witte korstvorming opgemerkt. De geofiele schimmel *Microsporum gypseum* werd geïsoleerd uit het letsel, waarna een vier weken durende topicale behandeling met enilconazole werd gestart. Twee maanden na de behandeling was het letsel volledig hersteld. Deze case-report laat vermoeden dat zomereczeem in combinatie met systemische corticosteroïdebehandeling als risicofactor kan optreden voor een dermatofyteninfectie. Verder wordt het belang van een correcte staalname, isolatie en identificatie van de oorzakelijke kiem bediscussieerd.

INTRODUCTION

Dermatophytosis or ringworm is a superficial skin infection caused by dermatophytes. These filamentous fungi invade keratinized tissues, such as nails, claws, hair and stratum corneum of humans and animals causing mild to severe, localized or diffuse infections. Dermatophytes are classified in the genera *Epidermophyton*, *Trichophyton* and *Microsporum*. They can also be divided into three ecological groups according to their natural host or habitat. Humans are the natural host of anthropophilic dermatophytes. Zoophilic dermatophytes have one or more animal species as natural host, but as most of these fungi can also infect humans, they should be considered zoonotic. Geophilic dermatophytes have the soil as main reservoir, which may act as source of infection for animals and humans (De Hoog et al., 2000).

Dermatophytosis is one of the most common skin diseases in horses (Pilsworth et al., 2007). During an epidemiological survey carried out in Italy (Perugia), a prevalence up to 9% was found in riding horses (mainly *T. equinum*, no *M. gypseum* isolates). In all cases, infection was associated with clinical signs (Moretti et al., 1998). Main clinical features in horses include alopecia, erythema and crusting. Lesions are often restricted to skin areas in contact with the saddle or other horse tack (Chermette et al., 2008; Wobeser, 2015). In the present case report, dermatophytosis in a horse due to the geophilic *Microsporum gypseum* is described.

CASE PRESENTATION

An eight-year-old Friesian gelding presented in autumn 2015 with a single, well-circumscribed, slightly

elevated, dry, crusted and alopecic lesion on the thigh (Figure 1). The horse had a history of extreme sweet itch, for which it had been treated with corticosteroids (dexamethasone, 30 mg/daily per os) during summer. At the time the lesion appeared, the horse was still under corticosteroid treatment and was wearing an eczema blanket, which completely covered the lesion. Based on the clinical aspect of the lesions, dermatophytosis was suspected.

Prior to sampling, the skin lesion was superficially decontaminated with a 70%-ethanol solution and air-dried to reduce the number of contaminants. Hairs were plucked and scales were scraped from the margin of the lesion using a sterile scalpel blade. Skin scrapings and hairs were inoculated at several points on Dermasel agar (Oxoid, Basingstoke, UK), supplemented with penicillin G (1000IU/ml) and streptomycin sulphate (1mg/ml). Plates were incubated at 25°C and checked daily for growth. To prevent overgrowth by saprobic fungi, suspected dermatophyte colonies were purified on Sabouraud dextrose agar (SDA) (Oxoid). The identification was deduced from macro- and microscopic features on SDA. Colonies were stellate, with a marked powdery aspect, cream-colored to cinnamon-tan with whitish aerial mycelium in the center of the colony and with a yellowish-buff to brownish verso (Figure 2). Microscopic examination of the purified culture revealed very abundant macroconidia and sparse microconidia (Figure 3). Macroconidia were septated, fusiform, thin- and rough-walled and arranged in dense clusters. Some macroconidia had a flagellated appendix (Figure 3A). Microconidia were sessile or stalked, smooth- and thin-walled and clavate (Figure 3B). Based on morphological features, the isolate was identified as *M. gypseum*. To confirm the diagnosis, the ITS1-5.8S-ITS2 ribosomal DNA region of the isolate was amplified using the panfungal primers ITS1 and ITS 4 [6] and sequenced. BLAST search in the ITS RefSeq database (NCBI) revealed a 100% sequence similarity with *M. gypseum*. Given the limited extent of the lesions, topical therapy was initiated with an enilconazol emulsion (Imaverol, Janssen Animal Health, Beerse, Belgium), once weekly, for four weeks. The lesion improved gradually and full recovery was achieved after two months.

Figure 1. Clinical aspect of a *Microsporum gypseum* infection in a Friesian horse, which presented as a single well-circumscribed and crusty lesion on the thigh.

DISCUSSION

In this report, a case of equine dermatophytosis due to the geophilic dermatophyte *M. gypseum* is described. The main etiological agent of dermatophytosis in horses is the zoophilic dermatophyte *Trichophyton equinum*, which is highly contagious among horses and may also infect humans (Chermette et al., 2008; White et al., 1990). Other zoophilic dermatophyte species, such as *Microsporum canis* (including the former *Microsporum equinum*) (De Hoog et al., 2000), *Trichophyton mentagrophytes* and *Trichophyton verrucosum,* are less frequently involved (Moretti et al., 1998; Cabañes et al., 1997; et al., 2000; Khosravi and Mahmoudi, 2003). Hitherto, infection due to *M. gypseum* has only occasionally been reported in horses (Khosravi and Mahmoudi, 2003; Tanner, 1982; Chiers et al., 2003).

To the authors' knowledge, there is no evidence of transmission of *M. gypseum* infections between horses, even though this could be theoretically possible, for example by common use of saddle blankets. Although transmission to humans from animals cannot be entirely excluded (Chermette et al., 2008; Romano et al., 2000; Romano et al., 2009), ringworm due to *M. gypseum* is not considered zoonotic. As *M. gypseum* lives saprobically in the soil, the outdoor environment constitutes the main source of infection. The risk of infection is higher for horses that are not stalled, living outdoor (Chermette et al., 2008).

Dermatophyte infections in healthy horses are considered to be self-limiting (Chermette et al., 2008; Wobeser, 2015; Scott and Miller, 2011). However, treatment of active infection is appropriate to advance healing of the lesions and to reduce spread of the infective arthroconidia into the environment (Chermette et al., 2008; Wobeser, 2015). When tackling infections due to zoophilic dermatophytes, antimycotic therapy may also help to avoid spread of the infection to other animals and humans.

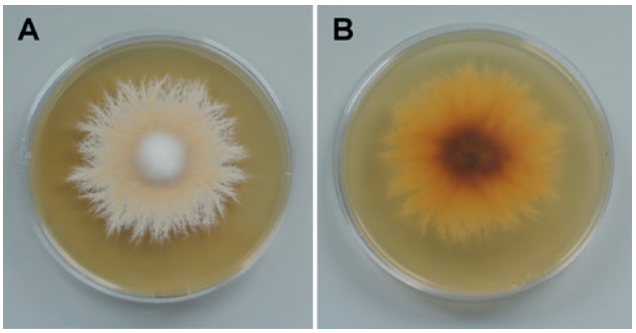

Figure 2. Macroscopical aspects of the purified *Microsporum gypseum* isolate, incubated on SDA for 7 days 25°C; A. recto; B. verso.

In the present case, the combination of sweet itch and the use of immunosuppressive drugs may have acted as predisposing factors. Sweet itch is a chronic, recurrent, seasonal dermatitis in horses caused by an allergic reaction to the bite of *Culicoides* spp. and is therefore often referred to as insect bite hypersensitivity (Van Grevenhof et al., 2007). The symptoms include intense pruritus, and the scratching may result in skin lesions and secondary infections. As a rule, the mane and tail are affected. However, in horses with severe signs, lesions may be spread over the entire body (Halldorsdottir and Larsen, 1991; Björnsdottir et al., 2006). So far, no connection between dermatophyte infection and sweet itch has been reported in the literature. Only Scott and Miller (2011) have mentioned that skin damage caused by ectoparasites (e.g. lice and biting flies) is probably important in the establishment and spread of dermatophytosis. The use of immunosuppressive drugs such as glucocorticoids, on the contrary, is known to entail an increased risk of developing fungal infections, including dermatophytosis (Chermette et al., 2008; Scott and Miller, 2011; Van Rooij et al., 2012; Guillot, 2013; Miller et al., 2013). Glucocorticoids interfere with first-line immune defences, mainly by suppressing the function of macrophages and inhibiting lysozyme synthesis (Panarelli, 1994; Papich, 2013).

Infections due to *M. gypseum* in horses may be more common than generally thought. Many cases of dermatophytosis in horses are not reported and the causal agent is often not identified. Isolation and identification of the causal agent may prove challenging. For example, to ascertain the cause of a dermatophytosis, proper specimen collection is of the utmost importance. The equine hair coat is a source for bacteria, saprobic moulds and yeasts that may complicate the isolation of causal dermatophytes by their fast (over) growth (Scott and Miller, 2011). Therefore, it is important that lesions are disinfected with 70% ethanol to reduce the occurrence of contaminants. Furthermore, scales should be scraped off and/or hairs should be plucked (not clipped) from the margins of the lesions. Even though commercial PCR kits are available to rapidly identify certain dermatophytes, including *M. gypseum*, they are often limited to the detection of only one species. Correct identification of the causal agent may help to identify the source of infection and to estimate the risk the infection spreads to other animals or humans.

CONCLUSIONS

To the authors' knowledge, this is the first case to suggest that sweet itch in combination with systemic corticosteroid treatment may act as predisposing factor for the development of dermatophytosis. In this paper, the importance of correct isolation and identification of the causal agent is also highlighted.

AVAILABILITY OF DATA AND MATERIALS

The data supporting the authors' findings are contained within the manuscript. Sequences are available in the EMBL/Genbank/DDBJ repository (accession number: LN886514; http: http://www.ebi.ac.uk/ena/data/view/LN886514). The *M. gypseum* isolate was deposited in the BCCM/IHEM repository (accession number: IHEM 26589).

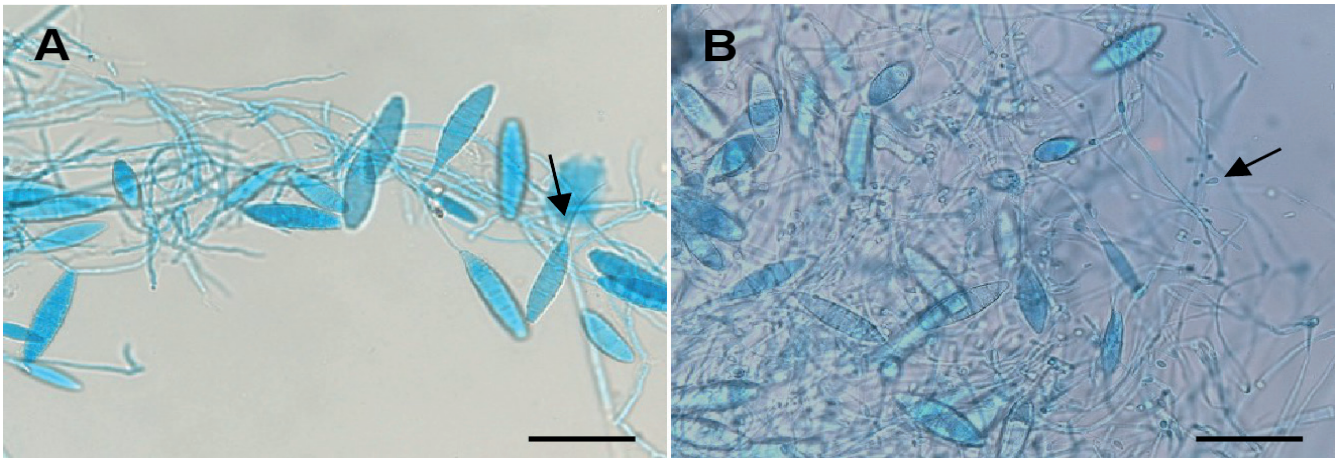

Figure 3. Microscopical morphology of the *Microsporum gypseum* isolate after purification and incubation on SDA at 25°C showing A. predominantly rough-walled, spindle-shaped macroconidia and a flagellated macroconidium (arrow) and B. sparse, club-shaped microconidia (arrow); scale bar: 50µm

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Mycobacterieel conjunctivaal granuloom bij een Chinese zaagbekeend (*Mergus squamatus*)

Mycobacterial conjunctival granuloma in a Scaly-sided merganser (Mergus squamatus)

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SAMENVATTING

Een vijf jaar oude, vrouwelijke Chinese zaagbekeend werd aangeboden met een chronische en wederkerende conjunctivitis van het linkeroog. Op klinisch onderzoek werden overvloedige traanvloei en een kazig nodulair letsel vastgesteld ter hoogte van de palpebrale conjunctiva. Bij autopsie werd een kazig, necrotisch beleg waargenomen ter hoogte van de linkerconjunctiva en de infraorbitale sinus, en er waren multipele granulomen in diverse interne organen aanwezig. Histologisch onderzoek toonde granulomateuze splenitis, hepatitis, arteritis, fibrinonecrotiserende enteritis, conjunctivitis en tevens amyloïdose van lever en milt aan. Bacteriologisch en moleculair onderzoek van de conjunctiva bij het levende dier toonde respectievelijk de aanwezigheid van een multiresistente *Escherichia coli* en *Chlamydia psittaci* aan. Deze bacteriën bleken van ondergeschikt pathogeen belang. Pre mortem werd echter geen cytologisch onderzoek of ziehl-neelsen (ZN)-kleuring op de histologische coupe van het conjunctivabiopt uitgevoerd. Bovendien werden pre mortem geen bijkomende onderzoeken, zoals bloedonderzoek en radiografie, uitgevoerd. De definitieve diagnose van een *Mycobacterium avium*-infectie ter hoogte van de conjunctiva en de inwendige organen werd pas bevestigd na autopsie door middel van een ZN-kleuring en polymerasekettingreactie (PCR)- analyse. Lokale en systemische behandelingen met antimicrobiële producten, zoals fluoroquinolonen, tetracyclinen en aminoglycosiden, waren niet succesvol.

ABSTRACT

A five-year-old, female scaly-sided merganser was presented with a chronic recurrent conjunctivitis of the left eye. Physical examination showed a caseous nodular lesion at the palpebral conjunctiva associated with excessive tearing. Necropsy revealed caseous necrotic debris at the left conjunctiva and infra-orbital sinus, but also multiple granuloma in several internal organs. Histological examination showed granulomatous splenitis, hepatitis, arteritis, fibrinonecrotic enteritis and conjunctivitis, and also amyloidosis of liver and spleen. Bacterial and molecular tests of a conjunctival swab in the live animal showed the presence of a multiresistant *Escherichia coli* strain and *Chlamydia psittaci*, respectively. These bacteria had only a secondary pathogenic role. No premortem cytological examination or Ziehl-Neelsen (ZN) staining of the histological section of the conjunctiva biopt or additional tests, such as blood test and radiographs, were performed. Finally, a *Mycobacterium avium* infection of the conjunctiva and internal organs was confirmed following necropsy by ZN staining and PCR analysis. Local and systemic antimicrobial treatments with fluoroquinolones, tetracyclines and aminoglycosides were unsuccessful.

INLEIDING

Conjunctivitis is een van de meest voorkomende oogproblemen bij gezelschapsvogels en wordt vaak gekarakteriseerd door een sereuze of seropurulente traanvloei. Differentiaal diagnostisch kan conjunctivitis bij vogels veroorzaakt worden door zowel infectieuze als niet-infectieuze agentia.

Trauma is een van de belangrijkste onderliggende niet-infectieuze oorzaken van conjunctivitis bij roofvogels, maar wordt ook frequent waargenomen bij andere vogelsoorten (Abrams et al., 2002). Daarnaast kan ook de aanwezigheid van een vreemd voorwerp zoals zand of grasaren, chemicaliën zoals ammoniakdampen, tumoren, hypovitaminose A, excessief vederverzorgingsgedrag (vaak door kooigenoot) of anatomische afwijkingen, zoals entropion, aanleiding geven tot conjunctivitis (Abrams et al., 2002; Miles et al., 2006; Souza et al., 2009; Gelatt en Plummer, 2017).

Parasitaire conjunctivitis kan bij vogels onder andere veroorzaakt worden door de nematoden *Oxyspirura spp.* en *Thelazia spp.,* de leverbot *Philophthalmus gralli*, de protozoa *Toxoplasma gondii*, *Trichomonas spp*. en *Cryptosporidium spp*. en myiasis (Abrams et al., 2002; Dubey, 2002; Elias et al., 2008; Lopez et al., 2010; Church et al., 2013; Dunham et al., 2016). Veel van deze parasieten komen echter zelden voor in West-Europa en infecties worden vaak uitsluitend waargenomen bij in het wild levende dieren of in het wild gevangen dieren.

Een bacteriële infectie is daarentegen de meest voorkomende oorzaak van conjunctivitis bij vogels. De diagnose van bacteriële conjunctivitis wordt bemoeilijkt omdat de normale conjunctivale microbiota bij vogels slechts in beperkte mate onderzocht zijn. Toch worden *Mycoplasma gallisepticum* en *Chlamydia psittaci* regelmatig geassocieerd met conjunctivitis bij watervogels (Abrams et al., 2002). In zeldzame gevallen kunnen ook *Mycobacterium spp.* oorzaak zijn van conjunctivitis bij vogels. Er werden reeds enkele gevallen gerapporteerd bij struisvogels, emoes, papegaaiachtigen, duiven, zangvogels en roofvogels, veroorzaakt door *Mycobacterium avium* (*M. avium*), *M. tuberculosis* of *M. genavense* (Washko et al., 1998; Korbel et al., 1997; García et al., 2001; Palmieri et al., 2013). Daarnaast werd ook nog een groot aantal andere bacteriën in verband gebracht met bacteriële conjunctivitis bij vogels, zoals *Haemophilus spp., Pasteurella multocida, Actinobacillus spp., Salmonella enterica* subsp. *arizonae, Escherichia coli, Streptococcus spp. Pseudomonas aeruginosa en Bordetella avium* (Zenoble et al., 1983; Devriese et al., 1988; Abrams et al., 2002). Ook infecties met schimmels (*Aspergillus fumigatus*) en gisten (*Candida spp*.) komen voor (Crispin en Barnett, 1978; Beckman et al., 1994). Een infectie met het aviaire pokkenvirus gaat meestal gepaard met unilaterale of bilaterale ulceratieve blefaritis met korstvorming, regelmatig gecombineerd met secundaire conjunctivitis en keratitis (Abrams et al., 2002). Herpesvirusinfecties bij vogels kunnen, naast talrijke andere klinische symptomen, ook conjunctivitis veroorzaken (Paulman et al., 2006; Shivaprasad en Phalen, 2012). Tenslotte worden ook nog talrijke andere virale oorzaken vermeld als occasionele oorzaak van conjunctivitis, zoals influenzavirus, paramyxovirus en adenovirus (Abrams et al., 2002).

In deze casus wordt een zeldzaam geval van conjunctivitis bij een eend beschreven geassocieerd met *Mycobacterium avium*.

Figuur 1. Kazige, nodulaire letsels ter hoogte van de palpebrale conjunctiva van het linkeroog.

CASUÏSTIEK

Een vijf jaar oude, vrouwelijke Chinese zaagbekeend (*Mergus squamatus*) van 680 gram werd aangeboden op de Faculteit Diergeneeskunde (UGent) met een unilaterale massa ter hoogte van het onderste linkerooglid. Het dier leefde samen met een mannelijke Chinese zaagbekeend en ongeveer twintig andere eenden van verschillende soorten in een volière van ongeveer 120 m², waarin vier vijvers aanwezig waren. Het water van deze vijvers werd continu gefilterd en de hokken werden regelmatig schoongemaakt. Het koppel werd na de start van de problemen afgezonderd van de andere dieren in een apart hok bij de eigenaar.

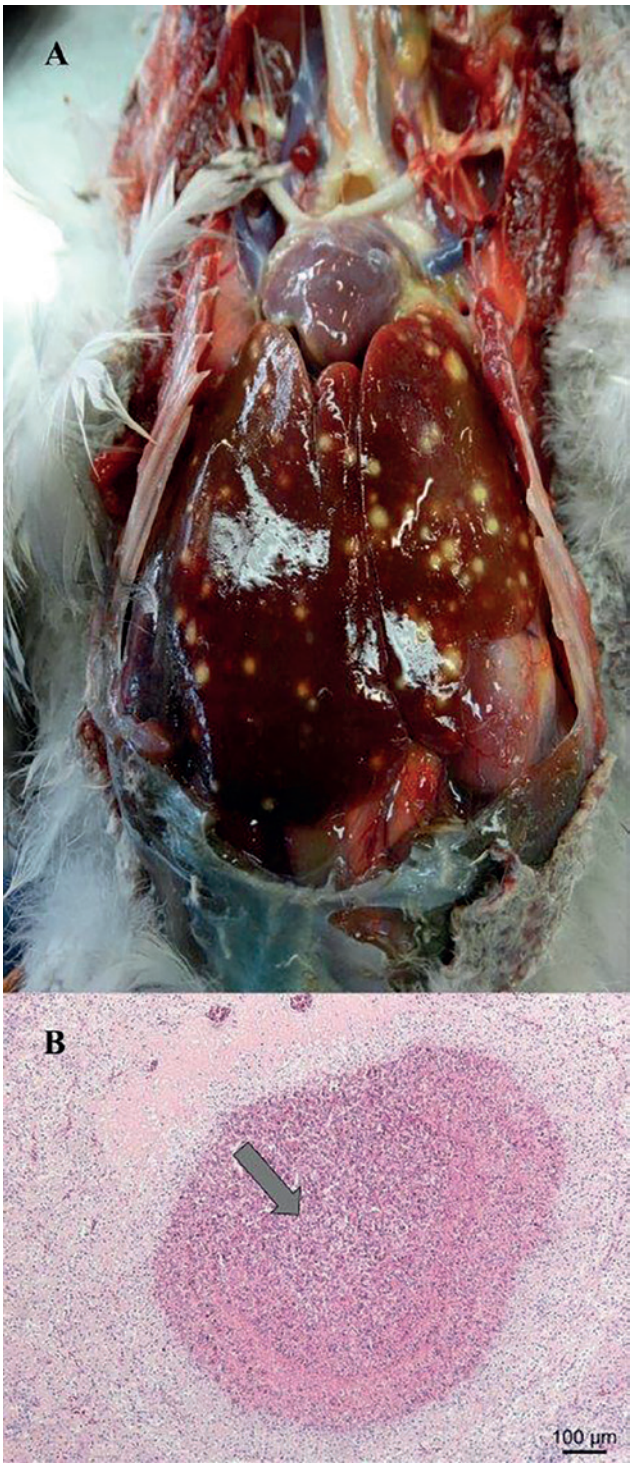
De massa ter hoogte van de conjunctiva werd enkele maanden vóór presentatie reeds opgemerkt door de eigenaar en werd toen succesvol behandeld met een lokale toediening van chlooramfenicol (Chlooramfenicol 1% + vitamine A oogzalf, Virbac, Barneveld, Nederland) en oxytetracycline oogzalf (Terramycine + polymyxcine B oogzalf, Pfizer, Brussel, België). Een maand vóór het dier op de Faculteit Diergeneeskunde (UGent) aangeboden werd, was de massa teruggekeerd en vertoonde ze een progressieve groei. Behandeling met enrofloxacine (Baytril 10%, Bayer Animal Health, Diegem, België) per oraal gedurende

1 week (20 mg/kg lichaamsgewicht (LG), 2 maal per dag (BID)) en ciprofloxacine oogdruppels (TID, ciloxan 3 mg/mL, Alcon, Puurs, België) voorafgaand aan de consultatie gaven geen beterschap. Ook bij het mannelijke dier van het koppel werd reeds enkele dagen overvloedige traanvloei opgemerkt.

Tijdens het algemeen onderzoek van het vrouwelijke dier werden een kazig, nodulair letsel ter hoogte van de palpebrale conjunctiva en overvloedige traanvloei opgemerkt ter hoogte van het linkeroog (Figuur 1). Cytologisch onderzoek van een conjunctivaswab van het linkeroog toonde bij hemacolorkleuring een overvloedige aanwezigheid van bacteriën, maar geen gisten of schimmelhyfen. Bacteriologisch onderzoek van een conjunctivaswab toonde een multiresistente *Escherichia coli* aan. Op basis van dit antibiogram werd de behandeling aangepast naar gentamycine-ooggel (TID, Clinagel-Vet, Ecupahar, Oostkamp, België). De eigenaar werd geadviseerd ook het mannelijke dier te behandelen.

Zeven dagen later was er geen beterschap bij het vrouwelijke dier. Bovendien was het vermagerd (LG 580g) en apathisch. Bij het mannelijke dier (LG 800g) werd wel beterschap opgemerkt. Omdat de behandeling niet succesvol was, werden er bijkomende diagnostische testen uitgevoerd. De PCR-test uitgevoerd op de conjunctivaswab voor *Chlamydia psittaci*, gaf een positief resultaat. De behandeling werd op basis van de resultaten van de PCR-test aangepast naar oxytetracycline-oogzalf (TID-QID, Terramycine + polymyxcine B oogzalf) en doxycycline per oraal (50 mg/kg LG, BID, Ronaxan 100mg, Merial, Diegem, België).

Na veertien dagen behandeling bleek de conjunctivaalzak echter opnieuw gevuld met een kazige substantie. Onder gasanesthesie (isofluraan + zuurstof) werd vervolgens een biopt genomen van de massa en van de conjunctiva voor verder histologisch onderzoek. Intraoperatief werd eveneens zoveel mogelijk etter weg gecuretteerd. Het histologisch onderzoek (hematoxyline en eosine (HE)- kleuring) van dit biopt toonde necrotisch weefsel aan met een uitgesproken infiltratie van heterofielen en lymfocyten. Gramkleuring toonde enkel groepjes staafvormige gramnegatieve bacteriën aan. De periodic acid-schiff (PAS)-kleuring was negatief voor schimmelhyfen. De algemene toestand van het vrouwelijke dier ging achteruit, gekenmerkt door lethargie en milde diarree. Enkele dagen later is het dier gestorven. Bij autopsie bleek de eend cachectisch (LG 595 g). De conjunctiva van het linkeroog was gezwollen en op de mucosa van het ooglid was een kazig/necrotisch beleg aanwezig. De linker infraorbitaal sinus was ook gevuld met die substantie. De dunne darm was gevuld met gas en vertoonde vaatinjecties. Het laatste deel van de dunne darm was sterk hemorragisch en necrotisch. De darmlussen waren onderling verkleefd. De longen waren gestuwd en oedemateus. Er was ook één granuloom aanwezig in de longen. De luchtzakken waren troebel en bevatten fibrineus exsudaat. Op de lever



Figuur 2A. Multifocale, granulomateuze letsels in de lever. B. Histologische afbeelding (HE-kleuring) van de lever gekarakteriseerd door de aanwezigheid van een granuloom (pijl) met accumulatie van fibrinonecrotisch materiaal met rondom gedegenereerde heterofielen, macrofagen en lymfocyten.

waren multifocale granulomateuze letsels en necrosehaardjes zichtbaar (Figuur 2). In de milt waren eveneens twee granulomateuze letsels te zien. Er werd ook flebitis van de linker vena jugularis opgemerkt.

Op cytologisch onderzoek (hemacolorkleuring) vertoonden de longen en nieren een ontstekingsbeeld,

voornamelijk gekarakteriseerd door een infiltratie van heterofielen maar ook van macrofagen en enkele lymfocyten. De lever vertoonde eveneens een ontstekingsbeeld en multifocale, blauwe, bolvormige structuren die suggestief waren voor *Aspergillus*-sporen. Mycologisch onderzoek was echter negatief. Ook in de milt werd er op het cytologisch onderzoek een ontstekingsbeeld waargenomen en was er massale aanwezigheid van gekleurde staafvormige bacteriën te zien. Cytologisch onderzoek van de inhoud van de dunne darm toonde blauwe, bolvormige structuren en een polybacteriële flora aan. Natief onderzoek van de inhoud van de dunne darm en caeca was negatief voor endoparasieten.

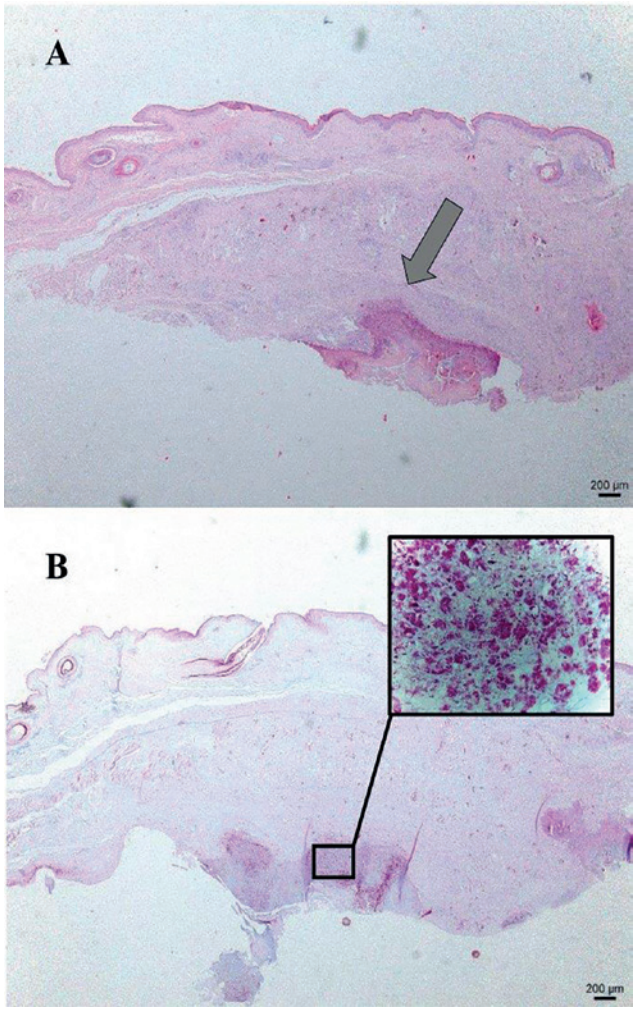
Er werd een histologisch onderzoek uitgevoerd van het ooglid (Figuur 3A), de lever, milt, darm, vena jugularis en de longen. Op HE-kleuring vertoonde de lever diffuus sterke atrofie van de leverbalkjes, accumulatie van een grote hoeveelheid homogeen, acellulair, eosinofiel materiaal (amyloïd) in de ruimte van Disse en verscheidene grote granulomen. Aan de mucosale zijde van het ooglid was er een uitgesproken ulceratie met een dikke serocellulaire korst op, die veel fibrine, gedegenereerde heterofielen en grote aantallen bacteriën (zeer kleine coccobacillen) bevatte. Meer heterofielen bevonden zich in de omgevende submucosa. In de milt waren multipele grote granulomen te zien. Rondom de sinusoïden was er amyloïd aanwezig.

In het darmlumen was een fibrineus pseudomembraan aanwezig waarin heterofielen zaten. De submucosa en het mesenterium waren diffuus oedemateus met een exsudaat van fibrine en heterofielen. In het lumen van de vena jugularis was een grote accumulatie van fibrinonecrotisch materiaal aanwezig met vele, zeer kleine, bacteriële kolonies. Rondom dit fibrinonecrotisch materiaal bevonden zich gedegenereerde heterofielen, macrofagen en lymfocyten. Talrijke confluente granulomen waren aanwezig in de longen, die de normale architectuur van het orgaan onherkenbaar maakten. De ZN-kleuring toonde aan dat er massaal veel zuurvaste bacteriën aanwezig waren in en rondom de granulomen (Figuur 3B). Histologisch onderzoek toonde aldus granulomateuze splenitis, hepatitis, arteritis, fibrinonecrotiserende enteritis, conjunctivitis, amyloïdose van lever en milt en een mycobacteriuminfectie van de conjunctiva, longen, lever en milt.

Een 16S ribosomaal RNA-gen gebaseerde PCR-test en sequenering (Tropisch Instituut, Antwerpen, België) toonden de aanwezigheid van *Mycobacterium avium* in de lever en conjunctiva aan.

DISCUSSIE

In deze casuïstiek wordt een geval beschreven van mycobacteriose bij een Chinese zaagbekeend die klinisch gekarakteriseerd wordt door de aanwezigheid van een palpebraal conjunctivaal granuloom, granulomateuze septicemie, lethargie en vermageren.



Figuur 3A. Granuloom (pijl) ter hoogte van de conjunctiva met accumulatie van fibrinonecrotisch materiaal (HE-kleuring). B. De ziehl-neelsenkleuring toont de massale aanwezigheid van zuurvaste bacteriën in en rond de granulomen aan.

Uitwendige letsels komen zelden voor bij mycobacteriose bij vogels. De enige afwijkingen die op klinisch onderzoek vaak vastgesteld worden, zijn vermageren met duidelijke atrofie van de pectorale spieren en het afnemen van de subcutane vetreserves (VanDerHeyden, 1997a; Shivaprasad en Palmieri, 2012). In een retrospectieve studie bij Psittaciformen werd vermageren bij 36,4% van de gevallen vastgesteld (Palmieri et al., 2013). Uitzonderlijk worden kleine nodulen in de huid waargenomen van enkele millimeters tot 1 cm groot, voornamelijk ter hoogte van de kop, hals of nek, maar soms ook ter hoogte van poten, vleugels, abdomen en rug. Hiernaast worden soms, zoals in dit geval, ook letsels aan de ogen waargenomen, zoals protrusie van één of beide ogen ten gevolge van een retrobulair granuloom, keratitis en kazige nodulen ter hoogte van de palpebrale en bulbaire conjunctiva, de ooghoek en het derde ooglid (Shivaprasad en Palmieri, 2012). In een studie van Palmieri et al. (2013) werden gezwollen oogleden slechts bij 4,7% van de gevallen van mycobacteriose bij papegaaiachtigen waargenomen. De macroscopische bevindingen bij

autopsie van de eend in de huidige casus, zoals kazige granulomen en spekachtige letsels ter hoogte van verschillende organen, zijn karakteristiek voor mycobacteriuminfectie (VanDerHeyden, 1997a; Shivaprasad en Palmieri, 2012). De waargenomen amyloidose van de lever is ook een typische bevinding bij chronische inflammatoire ziekten zoals mycobacteriose. Echter, aangezien Anseriformen zoals eenden een genetische predispositie hebben voor de ontwikkeling van amyloïdose, kan deze simultane bevinding ook toevallig zijn (Shivaprasad en Palmieri, 2012).

Omdat uitwendige letsels en pathognomonische klinische symptomen vaak ontbreken, is de ante-mortemdiagnose van mycobacteriose bij vogels moeilijk. Biochemisch bloedonderzoek toont soms milde aspecifieke afwijkingen aan van de leverenzymen en galzuren secundair aan diffuse weefselschade. Daarnaast is op hematologisch onderzoek vaak polychromasie, al dan niet samengaand met anemie waar te nemen. Bij sommige vogels is leucocytose aanwezig gekarakteriseerd door heterofilie, monocytose en lymfocytose (VanDerHeyden, 1997a). Op het cytologisch onderzoek werd in de lever en longen van de eend van de voorliggende casus ook een massale infiltratie van heterofielen, macrofagen en lymfocyten waargenomen. Een ZN-kleuring van de mest kan de aanwezigheid van zuurvaste bacteriën bij de intestinale vorm van mycobacteriose aantonen maar zelden bij andere vormen (VanDerHeyden, 1997a; Shivaprasad en Palmieri, 2012; Palmieri et al., 2013). Bovendien is de uitscheiding van de bacterie intermitterend. Radiografisch onderzoek kan de aanwezigheid van granulomen in de longen of de coeloomholte aantonen. Andere niet-specifieke bevindingen die waargenomen kunnen worden zijn hepatosplenomegalie, tympanie en verhoogde botdichtheid van het endost (VanDerHeyden, 1997a). Radiografisch onderzoek helpt de inwendige letsels te lokaliseren voor eventuele biopsie. Endoscopisch onderzoek van de coeloomholte is de beste methode om de nodige stalen te verzamelen van organen voor een definitieve diagnose. Eventueel kan een leverbiopt ook transcutaan of via coeliotomie genomen worden (VanDerHeyden, 1997a). Mycobacterium-granulomen zijn typisch wit tot bruin en rond van vorm, in tegenstelling tot aspergillose granulomen die vaak meer schijfvormig zijn. In de differentiaaldiagnose moet ook rekening gehouden worden met andere bacteriële granulomen (bijvoorbeeld ten gevolge van salmonellose, rodentiose of coligranulomatose) en abdominale tumoren, zoals lymfoma's. Een biopt dient genomen te worden voor verder onderzoek. Voor het cytologisch of histologisch onderzoek kan gebruik gemaakt worden van een standaardkleuring (bijvoorbeeld HE-kleuring) of een ZN-kleuring (Shivaprasad en Palmieri, 2012). In tegenstelling tot op histologisch onderzoek zijn mycobacteriën in de praktijk vaak te herkennen op cytologisch onderzoek als niet-kleurbare, staafvormige bacteriën op een standaardkleuring. In de huidige casus werd echter geen cytologisch onderzoek of ZN-kleuring op de histologische coupe van het biopt uitgevoerd. Er werden wel overvloedig gramnegatieve bacteriën waargenomen, vermoedelijk secundaire multiresistente *E. coli*, en geen schimmels. De definitieve diagnose kon in deze casus pas gesteld worden op basis van ZN-kleuring en PCR van de aangetaste organen na autopsie. Serologische testen, zoals de snelle draagglas-agglutinatietest en ELISA, zijn sterk diersoort-gerelateerd en zijn weinig specifiek, wat resulteert in veel valspositieve resultaten (Cromie et al., 1993; Amador et al., 2010). Bij een positieve tuberculinehuidtest of mantouxtest, waarbij een opgezuiverd, met hitte behandeld eiwitderivaat van *M. avium* of *M. tuberculosis* (0,05-0,1 mL, 2000 IU) intradermaal in de kinlellen wordt geïnjecteerd, ziet men na 48 uur een oedemateuze zwelling of een kleine nodule ter hoogte van de injectieplaats. Deze test is diagnostisch bij pluimvee. De werkzaamheid bij gezelschapsvogels is echter onbekend. Bovendien ontbreken dergelijke huidaanhangsels bij vele vogelsoorten (Fulton en Sanchez, 2008). De diagnose kan bevestigd worden door bacteriecultuur, maar de kiem groeit traag (VanDerHeyden, 1997a), waardoor de voorkeur naar moleculaire technieken gaat.

In geval van mycobacteriose wordt meestal geen behandeling ingesteld omwille van het zoönotische belang, de kostprijs en de therapieduur (12-18 maanden). Bovendien zijn er slechts weinig farmacokinetische en farmacodynamische gegevens bekend over het gebruik van antimycobacteriële geneesmiddelen bij vogels. De mycobacterie buitenmembraan bestaat uit een dikke hydrofobe barrière, wat de werking van hydrofiele antimicrobiële producten, zoals fluoroquinolonen, macroliden en aminoglycosiden beperkt. Uit empirisch onderzoek is gebleken dat *M. avium*- of *M. genavense*-infecties bij gezelschapsvogels behandeld kunnen worden met rifampicine, rifabutine, ethambutol, clofaziminine, ciprofloxacine, enrofloxacine, doxycycline, streptomycine, amikacine, azitromycine of claritromycine (VanDerHeyden, 1997b). Behandeling met fluoroquinolonen, tetracyclinen of aminoglycosiden gaf in deze casus geen verbetering. Vaak wordt er echter gebruik gemaakt van een combinatietherapie met bijvoorbeeld initieel rifabutine (56 mg/kg LG) en ethambutol (56-85 mg/kg LG). Later kan daar azitromycine (43 mg/kg LG) of claritromycine (85 mg/kg LG) aan toegevoegd worden. Bij vogels die slecht reageren op deze therapie kunnen nog fluoroquinolone, aminoglycoside en/of clofazimine (6-12 mg/kg LG) toegevoegd worden (VanDerHeyden, 1997b). Vanuit het standpunt van verantwoord gebruik van antibiotica in de diergeneeskunde en het zoönotisch aspect van mycobacteriose zou het gebruik van dergelijke behandelingsprotocollen beperkt moeten worden. Bij een uitbraak worden geïnfecteerde dieren beter geëuthanaseerd. De omgeving, voornamelijk de bodem en bodembedekking, vormt echter de meest belangrijke bron van infectie. Omdat de bacterie tot meer dan vier jaar lang kan overleven in de omge-

ving, is het frequent verwijderen van uitwerpselen en bodembedekking van primordiaal belang in de aanpak van de infectie (Fulton en Sanchez, 2008). Het is sterk aan te raden om gedurende meerdere jaren geen vogels te huisvesten in een besmette omgeving.

De aanwezigheid van *C. psittaci* ter hoogte van de conjunctiva bevestigd met PCR in de huidige casuïstiek, wijst niet noodzakelijk op een oorzakelijk verband met de waargenomen symptomen. Infecties met *C. psittaci* bij eenden verlopen vaak asymptomatisch, maar via massale fecale uitscheiding kunnen deze dieren de omgeving, inclusief het vijverwater, sterk besmetten (Gorham en Lee, 2016). *C. psittaci*-infecties worden meestal behandeld met doxycyline, chloortetracycline of enrofloxacine (Vanrompay et al., 1995; Perelman et al., 2013). Desondanks was er in deze casus weinig reactie op de antimicrobiële behandeling van *C. psittaci* en *E. coli*. Door het obligate intracellulaire voorkomen van chlamydia is de overdracht van antimicrobiële resistentiegenen beperkt. Antimicrobiële resistentie bij *C. psittaci* is nog onbekend. Wel werd tetracyclineresistentie reeds vastgesteld bij *Chlamydia suis* (Dugan et al., 2004; Joseph et al., 2016). In de voorliggende casuïstiek behoorde de geïsoleerde multiresistente *E. coli* tot de normale conjunctivale microbiota en/of was eerder van secundair pathogeen belang (Chalmers et al.,1984; Abrams et al., 2002).

Uit deze casus kan geconcludeerd worden dat mycobacteriose dient opgenomen te worden in de differentiaaldiagnose van chronische of terugkerende conjunctivitis bij eenden. Bijkomende testen, zoals ZN-kleuring en/of PCR van het aangetaste ooglid, ter detectie van een infectie met mycobacteriën zijn dan ook te adviseren. Daarnaast kunnen radiografie en/of endoscopie uitsluitsel geven over de eventuele aanwezigheid van granulomen in organen.

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Dierenweetjes

DIEREN TREUREN OM HUN DODE SOORTGENOTEN

'Ervaren olifantenexperts zijn het er over eens dat olifanten over een basisconcept van de dood beschikken. Net zoals wij het lichaam van een dood mens of een menselijk skelet herkennen, herkennen olifanten karkassen en skeletten van hun eigen soort. Ze ruiken aan de beenderen van hun doden, zelfs aan oude door de zon gebleekte botten, en ze strelen ze met hun slurf. Wanneer een olifant net gestorven is, raken andere olifanten vaak om beurten het lijk met hun achterpoten aan, bedekken het lichaam vervolgens met zand en stokken en houden de wacht. Intrigerend genoeg deden ze hetzelfde met lichamen van mensen die ze dood hebben gevonden, of die ze zelf hebben gedood. Chimpansees, gorilla's, sommige kraaiachtigen en dolfijnen brengen ook enige tijd met hun doden door, maar de meeste soorten in het algemeen niet.'

Iets ingekort overgenomen uit Morell V. (2013). *Hoe Dieren Denken*. Uitgeverij Balans, Amsterdam, p. 175. Oorspronkelijke titel: *Animal Wise*.

Luc Devriese

Perception, motivators and obstacles of biosecurity in cattle production

Perceptie, drijfveren en hindernissen van bioveiligheid in de rundveeproductie

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ABSTRACT

Farm biosecurity includes the prevention of disease transmission within and between farms. Two studies were set up to investigate what motivates and withholds farmers to implement biosecurity measures. The first study aimed to assess the perception of cattle farmers towards biosecurity and to identify possible reasons for its low application. This study consisted of a focus group discussion, of which the trends were used to develop a questionnaire for Flemish cattle farmers. Although the 91 participating farmers were familiar with several measures, they associated them with disease prevention rather than biosecurity. Nearly all farmers (98%) identified their herd veterinarian as their main source of information. Twenty percent and 32% of the respondents were convinced that the implementation of biosecurity would cost them more money and time, respectively. Finally, 80% of the farmers saw room for improvement of their herd, but indicated a need for practical information. The second study aimed to identify the key elements of twenty preselected biosecurity measures, motivators and obstacles for their implementation. They were judged by twenty-two cattle stakeholders, such as farmers, veterinarians and advisors, on feasibility, efficacy and return on investment. A box for parturition, a plan for rodent control and cleaning and disinfection of the stables scored highest overall. The lowest scoring measures were showering before entering the stables and an animal-free period of 24 hours. Their return on investment scored very low, since high investments are required. For a farmer to understand why biosecurity is beneficial, he must understand all risks and costs of disease and how biosecurity may reduce these risks and costs.

SAMENVATTING

Bioveiligheid omvat de preventie van ziektetransmissie binnen en tussen bedrijven. Twee aparte studies werden uitgevoerd om te onderzoeken wat veehouders aanzet of tegenhoudt om bioveiligheidsmaatregelen te implementeren. De eerste studie had tot doel de perceptie van rundveehouders rond bioveiligheid in kaart te brengen en de oorzaken voor de lage implementering te identificeren. Het eerste deel van deze studie bestond uit een focusgroepgesprek waarvan de trends gebruikt werden om een vragenlijst te ontwikkelen voor een enquête gericht tot Vlaamse rundveehouders. Hoewel de 91 deelnemende rundveehouders weet hadden van verscheidene bioveiligheidsmaatregelen, associeerden zij deze eerder met ziektepreventie dan met bioveiligheid. De bedrijfsdierenarts lijkt de voornaamste bron van informatie voor de veehouder (98%). Slechts twintig procent en 32% van de veehouders zijn ervan overtuigd dat de implementatie van bioveiligheid hen respectievelijk meer geld en tijd zou kosten. Tenslotte ziet 80% van de veehouders ruimte voor verbetering binnen hun bedrijf en geven ze aan nood te hebben aan praktische informatie. De tweede studie was gericht op de identificatie van hoofdpunten van twintig op voorhand geselecteerde bioveiligheidsmaatregelen, drijfveren en hindernissen aangaande de implementatie ervan. Ze werden door tweeëntwintig professionals uit de rundveesector, zoals veehouders, dierenartsen en adviseurs, beoordeeld op haalbaarheid, efficiëntie en return-on-investment. De afkalfstal, een knaagdierbestrijdingsprogramma en de reiniging en desinfectie van de stallen scoorden het hoogst. De laagst scorende maatregelen waren het douchen vooraleer de stallen te betreden en een 'diervrije' periode van 24 uur. De return-on-investment hiervan was heel laag, omdat daarvoor hoge investeringen noodzakelijk zijn. Om te begrijpen waarom bioveiligheid voordelig is, moet de veehouder een beeld krijgen van alle risico's en kosten van ziekte, en inzien hoe bioveiligheid risico's en kosten kan verminderen.

INTRODUCTION

The shift from curative to preventive veterinary medicine involves among others the implementation of biosecurity measures. By definition, biosecurity refers to all measures aiming to prevent pathogens from entering a herd and reducing the spread of pathogens within a herd (Lin et al., 2003; Villarroel et al., 2007; Derks et al., 2013; Laanen et al., 2013). Biosecurity is considered essential for the control of both epidemic (Heffernan et al., 2008) and endemic (Barrington et al., 2002 ; Callan and Garry, 2002) diseases. It is believed that biosecurity can contribute to a better animal health, better animal welfare, higher profits, higher effects of administered vaccines and lower selection for resistance by lower use of antibiotics and anthelmintics (Gunn et al., 2008; Brennan and Christley, 2013; Sarrazin et al., 2014; Nöremark et al., 2016).

However, the actual implementation of biosecurity measures is often low (Ribbens et al., 2008; Nöremark et al., 2010; Van Steenwinkel et al., 2011; Sarrazin et al., 2014). In Belgium, biosecurity measures are better established in pig and poultry production (Ribbens et al., 2008; Van Steenwinkel et al., 2011; Filippitzi et al., 2017), whereas in cattle production, there is much room for improvement of the biosecurity (Sarrazin et al., 2014). In a recent study, it has been shown that few biosecurity measures are undertaken by Belgian cattle farmers, thereby exposing themselves to the risk of disease transmission within and between farms (Sarrazin et al., 2014). Although basic biosecurity measures, such as farm-specific protective clothing and boots, are present in the majority of the farms, they appear to be insufficiently or incorrectly used. These findings raised questions about the reasons behind the limited implementation of biosecurity measures. Lack of information, a higher workload, more time loss or higher investments have been reported in the literature as reasons for this low implementation (Dargatz et al., 2002; Gunn et al., 2008; Brennan and Christley, 2013; Kuster et al., 2015; Nöremark et al., 2016). However, these do not seem applicable to the study of Sarrazin et al. (2014).

Two separate studies were set up to investigate what motivates and withholds farmers to implement biosecurity measures. The aim of the first study, consisting of a qualitative and quantitative part, was to assess the perception of cattle farmers towards the implementation of biosecurity and to identify possible reasons for the low level of application. A second study aimed to identify key elements of known biosecurity measures and the motivators and obstacles for their implementation judged by a range of cattle stakeholders.

MATERIAL AND METHODS

Study 1: Assessment of the perception of Flemish cattle farmers towards the implementation of biosecurity

This study consisted of a first, qualitative part in the form of a focus group and of a second, quantitative part in the form of a questionnaire based on the results of the first part.

Members of a local farmers association in Flemish Brabant were invited to participate in a focus group session to obtain a homogeneous, 'naturally occurring' group of professional cattle farmers, in which both dairy and beef cattle farmers were represented. During the focus group session, their attitude concerning the implementation of biosecurity on cattle farms was discussed, as well as advantages and disadvantages of biosecurity, possible improvements and their opinion on several specific biosecurity measures. The discussion was held in the native tongue of the farmers.

Participants were informed that all recorded data would be processed and analyzed anonymously before the start of the discussion. The discussion was led by a moderator, while another person took notes. The focus group consisted of eight female Flemish cattle farmers and the duration was aimed to be a maximum of two hours according to the guidelines of Kitzinger (1995). First, the participants were asked what they thought farm biosecurity was comprised of. Next, the interviewers explained their definition of farm biosecurity, i.e. the combination of all measures to prevent pathogens from entering a herd and to reduce the spread of pathogens within a herd, and brought eleven statements and open questions about biosecurity into the discussion (Table 1). Each of these were introduced to the participants in a neutral and objective way, with the help of a slide presentation. The participants were asked to express their opinion and talk about personal experiences in order to assess their knowledge about biosecurity, the implementation on-farm of specific measures and the possible difficulties of implementing these measures in practice.

The notes taken during the focus group discussion were anonymized and elaborated afterwards. The results of the focus group discussion were analyzed by summarizing trends across the topics that were discussed and applied to draft the survey for the second part of the study.

Based on the results of the focus group discussion, a questionnaire with five open questions (Table 2) and 18 semi-closed (13 questions with a 5-level Likert scale and five multiple choice questions listed in Table 3) was developed and conducted face-to-face during the fair for Flemish agriculture (Agriflanders) in January 2015. Subjects were randomly chosen and asked to participate anonymously. Only Flemish

Table 1. Eleven statements and open questions about biosecurity discussed during the focus group session.

1	What is your view on biosecurity and why?
2	Is biosecurity advantageous for the animals/farmer/…? Disadvantages?
3	Which disease present on your farm do you consider the most important?
4	Can biosecurity measures be helpful in handling this disease (less frequent, less severe)?
5	Research has shown that farmers consider keeping a closed farm (no purchase) and testing purchased animals as very important. However, in practice very little attention is devoted to these measures. Why? What is holding them back?
6	Do you quarantine newly purchased animals? And if so, why or why not?
7	On the majority of Belgian cattle farms farm-specific clothes and boots are present for visitors such as veterinarian, cattle salesmen, … Yet, they are rarely used.
8	One of the most 'forgotten' measures is the working from young animals to old, from healthy to sick.
9	What opportunities do you see to improve biosecurity in practice (in general or specific)? How can cattle farmers be encouraged to improve biosecurity on their own farm?
10	Who should participate in biosecurity on your farm?
11	What reasons do subjects give for not implementing biosecurity measures?

Table 2. Open questions during the survey.

1	Can you give a short description of the term 'biosecurity'?
2	Can you give a short description of the term 'animal disease prevention'?
3	Is it harder for cattle farmers to implement biosecurity on farm than for pig or poultry farmers (5-level Likert scale)? Why?
4	Describe how you execute quarantine on your farm?
5	Which biosecurity measures could be improved on your farm?

cattle farmers were invited to participate. The questions were limited to consider a time limit of five minutes per interview and the questionnaire was pretested. Responses for the open questions were categorized based on recurrent answers. The results of the questionnaire were analyzed using basic descriptive analysis in Microsoft Excel and the statistical package IBM® SPSS® Statistics 24.

Study 2: workshop with stakeholders in cattle production

A workshop was organized by the European Innovation Partnership for Agricultural Productivity and Sustainability (EIP-AGRI) in Brussels in January 2015 to identify the key elements of known biosecurity measures and the motivators and obstacles for their implementation. The participants evaluated twenty biosecurity measures by considering for each measure the expected efficacy in disease prevention, the expected feasibility and the expected return on investment (ROI) on a 5-level Likert scale (very low, low, neutral, high, very high) (Table 3). They were first asked to score the measures individually. Afterwards, participants of different backgrounds were asked to discuss their results in small groups. The results displayed in this article are the individual scores obtained from the participants. The results of the workshop were analyzed using basic descriptive analysis in Microsoft Excel. To each level of the Likert scale, a score was attributed: a score of -2, -1, 0, 1 and 2 for the levels very low, low, neutral, high and very high, respectively. For each of the twenty measures, the mean score was calculated based on the answers of the twenty respondents to obtain an average score for feasibility, efficacy and ROI. Additionally, an overall score per measure was calculated by making the sum of the respective scores of feasibility, efficacy and ROI. Finally, the measures were ranked based on these scores.

RESULTS

This paper is written in English while the focus group discussion was conducted in Dutch, the native tongue of the participating farmers. Although all quotes are translated as accurately as possible, nuances may still have gone lost in translation. Therefore, the original Dutch quote is noted after the English translation.

Study 1: Assessment of the perception of cattle farmers towards the implementation of biosecurity

Focus group discussion

Four of the participants were dairy farmers, one was a beef farmer and three had both dairy and beef cattle. The discussion lasted for an hour and a half. The results of the discussion are presented below. Statements are mentioned in bold, while pertinent quotes are shown in italics.

Definition of biosecurity and examples

The participants could not define biosecurity; yet, they were able to associate several aspects to the term, such as avoiding carry-over of diseases, hygiene and no visitors in the stables.

What is your view on biosecurity and why?

When explaining the definition of biosecurity as used by the investigators, the first reaction of the participants was that the term biosecurity is frightening. Moreover, they assessed the term as misleading, e.g. *"'bio' refers to biological agriculture" ("'bio'" doet denken aan biologische landbouw)*.

Nor did they link the word biosecurity to animal husbandry and felt that the term was not directly interpretable. They felt no involvement with the term and believed it refers to a process that takes place above them. As for the participants, animal disease prevention would be a more appropriate term.

Is biosecurity advantageous for the animals/ farmer/…? Disadvantages?

Nonetheless, with a better understanding of the term, they were convinced that the implementation of biosecurity measures could help in obtaining more healthy animals and consequently, might lower the veterinary costs. However, they seemed more focussed on the disadvantages. As possible disadvantages, the participants first mentioned more administration, for instance the registration of visitors. Secondly, they fear more rules and inspections; thirdly and most important according to the participants, the possible time-consuming aspect.

One of the participants believed it is harder in case of cattle to implement biosecurity than for other species, while another participant, who kept both poultry and cattle, mentioned it to be mainly an adjustment of behavior:

"Actually, it concerns automatic actions we as cattle farmers are not used to. It is mainly a mindset."
"Dat zijn automatismen die we niet gewoon zijn als rundveehouders. Het is een kwestie van instelling."

Which disease do you consider to be the most important on your farm? Can biosecurity measures be helpful in handling this disease (less frequent, less severe)?

Multiple participants stressed the importance of calf diarrhea in the calving period, with many calvings in a short period. In fall and early winter, the biggest fear is bovine respiratory diseases. Also bovine viral diarrhea (BVD) was mentioned. Some farmers had already experienced an outbreak or the presence of carriers despite attempts to eliminate the disease. Scabies is a disease, which is more important for beef cattle farms.

When mentioning the cleaning of boots, the participants asked themselves how far they must go in executing particular biosecurity measures.

"How far should we go? If sick animals are the last to be treated in the morning, are the pathogens of the morning still present on our boots and clothes in the evening? Should we change clothes then?"
"Wat voor nut heeft het als je bijvoorbeeld 's morgens de zieke dieren als laatste behandelt? Zijn de ziektekiemen van 's morgens, 's avonds nog aanwezig op je kleren en laarzen? Moet je dan ook van kleren wisselen?"

"What can you demand of your visitors without nagging?"
"Hoeveel kan je van je bezoekers verlangen zonder vervelend over te komen?"

"Perfectly shielded animals are more sensitive as they have no immunity to any disease."
"Perfect afgeschermde dieren zijn gevoeliger voor ziekte omdat ze geen immuniteit hebben."

"We can hardly leave our clothes outside next to the hutch of a sick calf."
"We kunnen die kledij toch niet buiten laten liggen bij die ene iglo met een ziek kalf?"

The participants argued that a strict separation between sick and healthy animals is practically not feasible; they also mentioned that they do not have physical contact with healthy animals and only treat the sick animals. They considered wearing gloves as an effective and feasible measure.

As a concluding remark, they stressed the need of information about simple biosecurity measures.

Research has shown that farmers consider a closed farm (no purchase) and testing purchased animals very important. Yet, in practice, very little attention is paid to these measures. Why? What is holding them back?

Although the participants knew and agreed with the principle, they argued that purchase is sometimes necessary. For example, animals need to be bought if there are not enough replacement heifers or in case new genetics need to be introduced on the farm.

Concerning the testing of purchased animals, trust in the purchase protocol of the Flemish animal health service (Diergezondheidszorg Vlaanderen) is high amongst the farmers. However, it is not clear which tests are compulsory. The participants suggested that it would be better to test the purchased animals on the farm of origin in order to avoid infected animals to be entered on a farm.

"There are several packages available, we always take the broadest one. Then you are sure everything has been tested."
"Er zijn verschillende pakketten in de aanbieding. Wij kiezen steeds voor het meest uitgebreide. Dan heb je alles getest."

"Are there many farmers who don't test at purchase ?"
"Zijn er nog veel bedrijven waar het aankoopprotocol niet uitgevoerd wordt?"

"Isn't it compulsory to test at purchase?"
"Is het aankoopprotocol niet verplicht?"

Next to ignorance, the cost of the purchase protocol seemed to be the main reason for not testing. Maybe, some farmers do not know that tests at purchase are financially supported.

Do you quarantine newly purchased animals? And if so, why or why not?

Quarantine, defined as a separation of newly purchased animals without possibility of direct contact with own animals, is quickly dismissed with the argument that there is no space available for separation. The question is raised what distance is sufficient.

"If there is no small, separate stable available at that moment, you will not invest in a separate quarantine stable."
"Als er op dat moment door omstandigheden geen klein apart stalletje ter beschikking is, dan ga je ook niet investeren in een aparte quarantainestal."

A quarantine duration of three weeks is considered very long. Especially for dairy cows in lactation, it is considered impossible as they need to go to the milking parlor. All participants realized this is a violation of the quarantine, but minimize the problem:

"… but that's only for a very short period of time. In that time, only little contact is possible and disease can't possibly be transmitted?"

"… maar dat is maar heel kort. Op zo'n korte tijd is er slechts weinig contact mogelijk en kan ziekte toch niet overgedragen worden?"

Animals returning, e.g. from competitions, are not placed in quarantine. The risks in that case were again minimized, even if the farmer realized that participation in competition is not advisable.

On the majority of Belgian cattle farms, farm specific clothes and boots are present for visitors, such as veterinarian, cattle salesmen, etc. Yet, they are rarely used.

The group is reluctant to ask professional visitors, i.e. herd veterinarians, artificial insemination technicians, cattle salesmen) to use herd specific clothing. As a first reason, the farmers mentioned that since veterinarians have a higher education, they should propose themselves to use herd specific clothing. Secondly, they believed it is hypocritical to demand visitors to use herd specific clothing, while the farmers themselves do not implement specific biosecurity measures, e.g. quarantining cattle returning from a fair.

"But can I really be angry at my vet if he doesn't wear my farm specific boots, if I violate biosecurity principles myself?"
"Kan je commentaar leveren op het feit dat de dierenarts de bedrijfseigen laarzen niet gebruikt als je zelf inbreuken pleegt met betrekking tot bioveiligheid?"

Both herd specific boots and a disinfection bath are compulsory by a quality control label (IKM). However, both are rarely used (correctly) by the farmers adhering to the program. Farmers need to have boots (and clothes) in multiple sizes, and a disinfection bath is considered to lose its effectiveness very quickly.

Herd specific clothes are considered cumbersome to put on and participants understand that visitors do not wish to wear them. For this reason, they prefer an overcoat or coveralls.

They concluded it is not easy for them to execute these measures consistently.

"Our farm lay-out is a hindrance to efficiently use herd specific boots and clothes: there are separate stables and if the stable to visit is too far from the sanitary transition zone, the visitors will not make the effort."
"Onze bedrijfsstructuur staat een efficiënt gebruik van de bedrijfskledij in de weg: er zijn verschillende stallen en als de stal waar de bezoekers verwacht worden te ver van het omkleedlokaal gelegen is, dan doen ze de moeite niet meer."

Another important biosecurity principle is working from young animals to old, from healthy to sick.

Table 3. Questionnaire results (%) for 13 semi-closed questions with a 5-level Likert scale (n=91).

Question	Likert scale[a]				
	1 Strongly disagree	2	3	4	5 Strongly agree
I am familiar with the term 'biosecurity'	8[b]%	34%	25%	32%	1%
I am familiar with the term 'animal disease prevention'	0%	1%	8%	85%	7%
The term 'biosecurity' is correct term for the concept (after explanation of the concept)	9%	41%	11%	40%	0%
The term 'biosecurity' scares me off	1%	40%	15%	43%	1%
As a farmer I feel responsible for 'biosecurity'	0%	4%	9%	79%	8%
As a farmer I believe that 'biosecurity' costs me more money than it raises	1%	59%	20%	18%	2%
As a farmer I believe that 'biosecurity' costs me more time and effort than it raises	0%	52%	17%	32%	0%
I have sufficient information to apply 'biosecurity' well in practice	1%	29%	19%	51%	1%
I am willing to pay for veterinary advice on 'biosecurity'/'animal disease prevention'	1%	44%	28%	26%	1%
As a cattle farmer it is more difficult to apply biosecurity well than as pig/poultry farmer	1%	39%	13%	44%	3%
I dare to demand visitors to use herd specific clothing	0%	19%	14%	60%	7%
A quarantine period of 3 weeks cannot be obtained on my farm	6%	22%	13%	45%	13%
I believe that the level of 'biosecurity' can be improved on my farm	0%	8%	12%	79%	1%

[a]1 = Strongly disagree; 2 = Disagree; 3 = Neutral; 4 = Agree; 5 = Strongly agree
[b]Due to rounding the percentages may not exactly sum up to 100%

The participants realized the importance of this measure and try to work accordingly. However, they raise some practical issues. For instance, they need to milk adult cattle before being able to feed that milk to the calves. Secondly, the farm lay-out is not always arranged appropriately to follow working lines; and last but not least, they believe working lines take more time. Time management and saving time are considered very important.

"*We love our job, but don't practice it for fun*"
"We doen ons werk graag, maar we doen het niet voor ons plezier."

What opportunities do you see to improve biosecurity in practice (in general or specific)? How can cattle farmers be encouraged to improve biosecurity on their own farm?

When discussing possible ways to improve the implementation of biosecurity in cattle farms, the group stressed the urgent need of additional practical and persistent support and information. This information needs to be practical and should be repeated. How the information is to be spread remains a point of discussion.

The preferred way to obtain this information is through the herd veterinarian. However, the participants remarked that their herd veterinarian has very little time to give preventive advice. Furthermore, they do not expect him/her to be aware of all possible information. They also believe that the herd veterinarian sometimes does not observe certain problems on a farm, as he visits the farm too often. Some participants prefer a more specialized consultant.

"*Our veterinarian is able to solve problems, but is not able to prevent them yet.*"
"Onze dierenarts lost problemen op, maar hij kan ze nog niet voorkomen."

"When trying to solve a farm-specific problem, external advice is appropriate, as the herd veterinarian is blind for some evident problems and does not have the time to deal with them. Herd health management should be an external affair."
"Bij een bedrijfsprobleem is advies van een derde partij aangewezen, want de bedrijfsdierenarts zit er te kort op en is blind voor sommige evidente problemen en heeft er bovendien geen tijd voor. Bedrijfsbegeleiding dient extern te gebeuren."

Furthermore, it is not clear whether there should be paid for veterinary preventive advice. When the

comparison is made to a doctor's appointment, the participants agreed that it is normal to pay for preventive advice.

"Disease prevention is something you might expect from your herd veterinarian, because he has the knowledge. However, because of the present high-pressure structure of the system, it is something you should not expect, because he does not have the time."

"Ziektepreventie is iets wat je van een bedrijfsdierenarts zou kunnen verwachten omdat hij/zij de kennis bezit. Anderzijds mag je van hem/haar geen ziektepreventie in dit veel te druk, huidig systeem verwachten wegens tijdsgebrek."

Who should participate in biosecurity on your farm?

The answer to this question was exceedingly clear: all visitors who have contact with the animals, should participate in biosecurity on the farm. Unexpected visitors, especially those with high education, can cause irritation when they do not take the available measures.

What arguments do visitors cite when they do not want to participate?

Some visitors do not believe in the usefulness of certain measures or believe it to be redundant as the farmer does not keep to all principles of biosecurity himself. Lack of time is also a frequently cited reason. Moreover, although they are not unwilling to participate, it is hard to habituate oneself to the measures. Other cited reasons were the absence of the correct size or brand of boots and clothing.

Questionnaire results

Ninety-one face-to-face interviews (25% dairy farmers, 43% beef and 32% mixed) were conducted (Table 3). All respondents originated from Flanders and the majority were male (88%).

Although the Flemish cattle farmers were familiar with several specific biosecurity measures, such as quarantine and the use of herd-specific clothing, they did not associate these measures with the term biosecurity. They tended to relate the term biosecurity to food safety (23.0%) and ecological farming (21.0%) or they had no idea what this concept meant (48.0%). Only 5.5% of the definitions provided by the participants could be considered correct. The farmers seemed to be more familiar with the term 'animal disease prevention' as they mentioned prevention of disease, control of BVD, IBR, neosporosis and other diseases and vaccination when defining this term. However, two of the participating farmers described disease prevention as the use of antibiotics. The type of farm did not seem to have influence on the knowledge of these terms.

Out of 38 participants who claimed not to be familiar with the term 'biosecurity', 65.8% followed working lines, 31.6% did not purchase cattle and 55.3% quarantines purchased animals (yet, only 40.4% had a separate quarantine stable and 28.4% of the farmers thought a quarantine period of three weeks is feasible). This shows that farmers indeed have more knowledge of separate biosecurity measures than of the term 'biosecurity' itself.

The majority of the farmers (87.0%) felt responsible for the implementation of biosecurity on their farm, and 52.0% of the respondents indicated to dispose of sufficient information to implement biosecurity adequately. Nearly all farmers (98.0%) identified their herd veterinarian as the main source of this information.

However, only 45.0% of the farmers is willing to pay their herd veterinarian for preventive advice. Forty-seven percent believed that it is more difficult to implement biosecurity for cattle farmers than for pig and poultry farmers, since cattle are pastured (30.0%) and cattle farms are more often visited (13.0%). Whether or not willing to pay for advice differed according to the preferred source of information: 100% of the participants who preferred an external source in combination with their herd veterinarian is willing to pay for advice, whereas only 23.6% of those expecting advice from the herd veterinarian only, is willing to pay.

Nonetheless, 80.0% and 78.0% of the respondents were convinced that the implementation of biosecurity would not cost them money nor require more time and effort, respectively. Of all the farmers, 80.0% saw room for improvement of the biosecurity level in their herd. Proposed biosecurity measures are quarantining purchased cattle, disinfection and herd specific clothing. However, 13.0% of these farmers first wanted to gain information before answering the question and 19.5% had no idea what to improve.

Results EIP-AGRI workshop

Twenty-two participants involved in cattle production, including farmers or their representatives, researchers, farmer advisors, practicing veterinarians, animal health services, representatives of trade, industry, government or EU from twelve European countries attended the workshop.

Scoring highest for the overall score is the presence and use of a separate box for parturition on the farm (Table 4 and Figure 1). In the second rank for the combined score, a systematic plan for rodent control can be found. A proper cleaning and disinfection of the stables are ranked third.

Using a separate box for parturition also returns in the single score for efficacy in the first rank. Proper cleaning and disinfection of the stables after each production round were considered very efficient (second rank), yet less feasible (shared sixth rank). Similarly,

Table 4. Ranking of perceived efficacy, feasibility, return on investment and an overall score for 20 biosecurity measures (for cattle). A ranking is provided for each measure and presented in bold for the top 5. A measure with score '1' was judged the highest, a measure with score '20' was judged lowest.

Biosecurity measure	Ranking			
	Overall score	Efficacy	Feasibility	Return on investment
A separate and dedicated box for parturition is available and used only for this purpose	**1**	**1**	**3**	**1**
A systematic plan for rodent control is installed	**2**	6	**1**	**3**
Proper cleaning and disinfection of the stables	**3**	**2**	7	**2**
All vehicles for animal transport are empty and cleaned and disinfected before entering the premises	**4**	**4**	6	**4**
No contact with animals from other farmers is possible through pasture contact	**5**	**3**	**5**	**5**
Dead animals are stored at a specific designated location away from the stables	6	8	**2**	6
All used equipment is stable specific	7	7	8	7
If animals are bought they are always kept in a quarantine stable for at least 4 weeks	8	**5**	11	11
Disposable or herd specific clothing and footwear required before entering the stables	9	9	10	9
Hygiene lock (specific designated changing and hygiene room for persons) available before entering the stable	10	13	13	14
Different age groups are strictly separated in different compartments	11	11	14	10
If animals are bought, they always come from the same supplier	12	10	17	8
Chemical and microbiological analysis of drinking water (at the source and the end 13 f the drinking line) at least once a year	16	**4**	16	
No pets allowed in the stables	14	14	12	12
Employees are not allowed to work on other cattle farms	15	12	9	13
A systematic plan for insect control is installed	16	17	15	17
Strict working lines (starting with the youngest animals and working towards the oldest animals) are used	17	18	16	15
Clear separation of the farmyard in a dirty and clean (no access for vehicles, persons or animals from outside the herd) area	18	15	19	18
A minimum "animal-free" period of 24 hours before entrance to the stables is allowed	19	19	18	19
Showering required before entrance to the stables	20	20	20	20

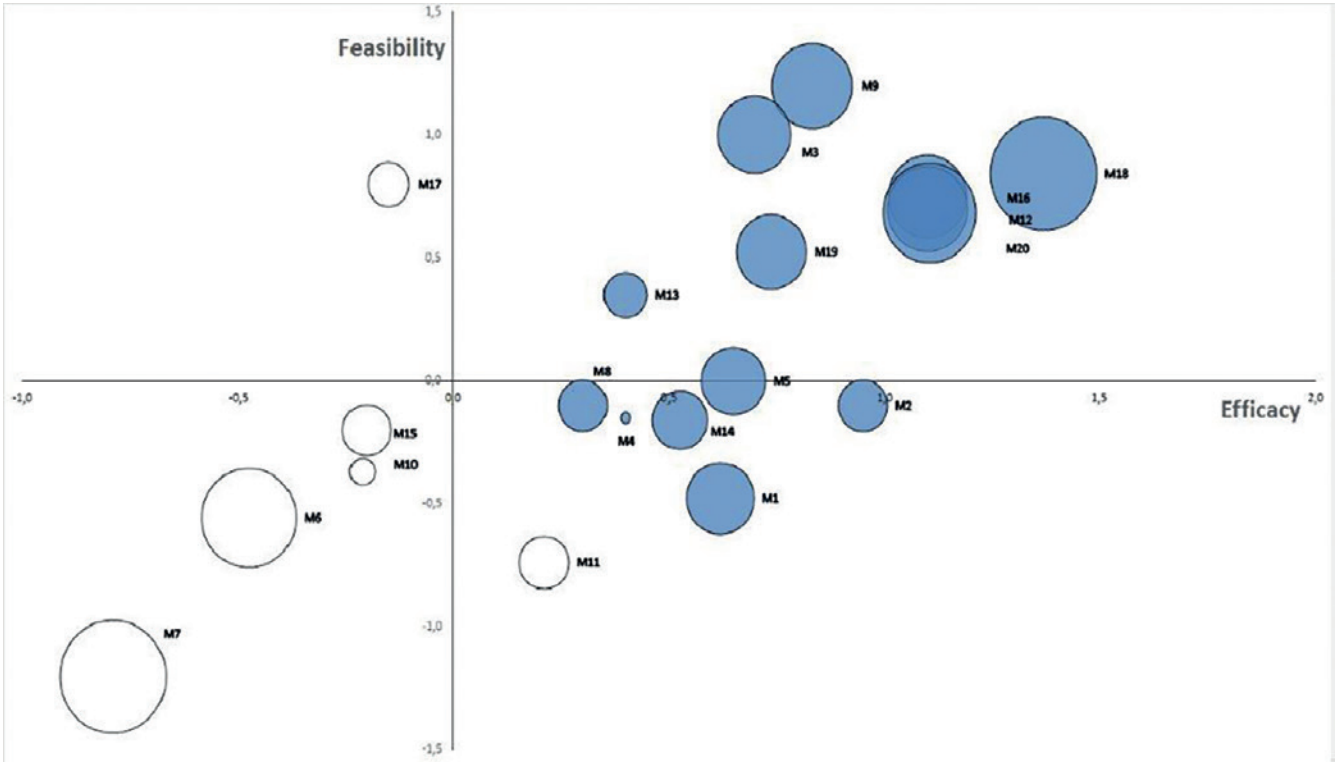

Figure 1. Visual representation of the average scores of the biosecurity measures for estimated efficacy (x-axis), feasibility (y-axis) and return on investment (bullets) based on a 5-level likert scale (-2: very low, -1: low, 0: neutral, 1: high, 2: very high). Return on investment is represented by the color and size of the bullets: a blue bullet denotes a positive value for return on investment, while a white bullet denotes a negative value for return on investment. A larger bullet size denotes a higher absolute value for return on investment.

Measure

- **M1 If animals are bought they always come from the same supplier**
- **M2 If animals are bought they are always kept in a quarantine barn for at least 4 weeks**
- **M3 Dead animals are stored at a specific designated location away from the barns**
- **M4 Hygiene lock (specific designated changing and hygiene room for visitors) available before entering the barn**
- **M5 Disposable or herd-specific clothing and footwear required before entering the barns**
- **M6 A minimum animal-free period of 24 hours is required before entrance of any visitors to the barns**
- **M7 Showering required before entrance to the barns**
- **M8 No pets allowed in the barns**
- **M9 Systematic plan for rodent control is installed**
- **M10 Systematic plan for insect control is installed**
- **M11 Clear separation of the farmyard in a dirty and clean (no access for vehicles, persons or animals from outside the herd) area.**
- **M12 All vehicles for animal transport are empty, cleaned and disinfected before entering the premises**
- **M13 No employees who are also working on other cattle farms**
- **M14 Different age groups are strictly separated in different compartments**
- **M15 Strict working lines (starting with the youngest animals and working towards the oldest animals) are used**
- **M16 Contact with animals from other farmers is not possible through pasture**
- **M17 Chemical and microbiological analysis of drinking water (at the source and the end of the drinking line) at least once a year**
- **M18 A separate and dedicated box for parturition is available and used**
- **M19 All used equipment is holding specific**
- **M20 Proper cleaning and disinfection of the barns after every production round**

mandatory cleaning and disinfection of vehicles before entering the premises of the farm are ranked third for efficacy.

A rodent control plan was judged the most feasible of all biosecurity measures. A carcass storage away from the stables scores the second rank for feasibility, but has a lower score for efficacy (shared seventh). In the third rank, a separate box for parturition returns.

ROI seems to be judged similarly to efficacy and feasibility as the two highest judged measures for efficacy score the same ranks in ROI (a separate box for parturition first, cleaning and disinfection after each production round second). Third highest for ROI is the biosecurity measure considered most feasible, a rodent control plan.

The common lowest scoring measure is a required shower before entering the stables. This measure scored rank twenty in all categories. The measure considered to be second lowest (rank eighteen for feasibility and rank nineteen in the other categories) is

the obligation of an 'animal-free' period of at least 24 hours before access to the stables is allowed. Their ROI is lowest of all, since high investments may be required to implement them.

DISCUSSION

In this paper, the results of two studies concerning the perception of biosecurity and biosecurity measures by cattle farmers and stakeholders are described. Both studies show that their knowledge of biosecurity principles can still be improved. The participants in both studies displayed a positive attitude towards biosecurity, but not necessarily a positive view on all biosecurity measures. In the first study, qualitative (focus group) and quantitative (questionnaire) research was combined to describe a possible thresholds for a good implementation of biosecurity measures in cattle farms from a farmers' point of view. Quantitative research is frequently used in veterinary medicine, while qualitative research is often criticized for not having enough scientific rigor (Krefting, 1990). Although in qualitative research, the participants are not assumed to be representative for the population, the obtained results can be useful to better understand the subject (Christley and Perkins, 2010). By using a combination of both types of research, the study has a higher validity and reliability. Qualitative research provides accurate, yet broad information, raising the validity of the research. When quantified, the more important elements can be identified, improving reliability. All participating farmers in the focus group discussion were female, which might create a misrepresentation of the results as most recorded farmers seem to be male (88% during the survey). However, most farms in Belgium are family businesses where the partners contribute equally to the farm. This was the case for the members of the focus group discussion. Results from the focus group discussion and the survey both converge and diverge on certain subjects; yet, it is not clear whether this is caused by the gender distribution of the participants or not. Many other differences, such as differences in posing questions or more specific questions are possible causes.

For an overall evaluation of biosecurity measures, as a sample of convenience during the EIP-AGRI days, the sample size of the present workshop was too small to draw conclusions on the application of biosecurity measures in the represented European countries. Nevertheless, the present evaluation may again serve as an indication of why or why not farmers are motivated to implement certain biosecurity measures. Moreover, it must be taken into account that the participants of this survey were not exclusively farmers; hence, the results of this study cannot be used to represent the farmers' opinion. The three criteria judged during this study were chosen as farmers seemed to consider them as crucial before implementing biosecurity measures. Feasibility was considered to have influence on the farmers' motivation as some measures were perceived impractical or impossible (Garforth et al., 2013). If farmers do not believe a measure to be important (efficient) or to have a positive ROI, they might not implement it. Unfortunately, evidence is often lacking (Alarcon et al., 2014).

During the first study, the participants found the term biosecurity frightening and did not feel involved with it as they thought biosecurity to be discussed on a higher level, i.e. governmental level. Participants were very familiar with the term disease prevention. If biosecurity would be introduced as part of disease prevention, perhaps farmers would be less suspicious and more receptive to the term.

Yet, those not comfortable with the term biosecurity in both parts of the study, had already implemented various biosecurity measures without knowing the measures concerned biosecurity. Hence, although they did not seem familiar with the term itself, they seemed to be familiar with some principles of biosecurity used and ranked during the second study.

The presence of a maternity pen was most highly judged of all measures and was also considered important by the participants of the first study. Although a maternity pen is often incorrectly used or not present (Sarrazin et al., 2014), in the literature, the maternity pen has been shown to be a highly profitable measure. Mee (1991) described a decrease of calf mortality rate and Curtis et al. (1988) observed a reduction in the occurrence of calf diarrhea when calves were born in a maternity pen. It has often been reported as important in avoiding perinatal calf mortality, morbidity (Svensson et al., 2003; Mee, 2004; Mee, 2008) or infection with several calf diseases (salmonellosis, cryptosporidiosis, respiratory diseases) (Garber et al., 1994; Svensson et al., 2003; Fossler et al., 2005a; Fossler et al., 2005b).

The importance of a rodent control program was mentioned during the first study, as the role of rodents as vectors and carriers of leptospirosis (Endepols et al., 2003; Sarrazin et al., 2017), salmonellosis (Tablante Jr and Lane, 1989; Warnick et al., 2001; Sarrazin et al., 2017) and other diseases (Endepols et al., 2003; Sarrazin et al., 2017) has been clearly proven. However, this measure was judged feasible and less efficient, possibly because farmers do not always consider a rodent infestation as a problem (Leirs et al., 2004); they use a subjective threshold to implement a rodent control program (e.g. visible confirmation of the infestation) (Endepols et al., 2003; Meerburg and Kijlstra, 2007) or do not execute the implemented plan properly (Endepols et al., 2003). The participants of the second survey were aware of the diseases rodents may transmit as a rodent control program scored high in all criteria. An insect control program on the other hand was ranked amongst the lowest of biosecurity measures, nor was it stressed during the first study. This may be considered strange as, next to skin

damages and irritations insects may cause, they may transmit multiple diseases, such as summer mastitis (Bramley et al., 1985), moraxellosis (Postma et al., 2008), besnoïtosis (Gutiérrez Expósito et al., 2017) and vector borne diseases like bluetongue (Tatem et al., 2003), lumpy skin disease (Tuppurainen and Oura, 2012) and Schmallenberg disease (Garigliany et al., 2012).

The two lowest scored measures, i. e. showering and an animal-free period, have been hardly reported in the literature concerning cattle biosecurity. Even in pig production, where biosecurity is more established, the level of implementation of these measures is low (Ribbens et al., 2008). The problem of time and investment may be raised as an obstacle for the implementation of these two measures. Additionally, it should be taken into account that farmers may not like to ask visitors to take a shower as it might be regarded as an invasion of privacy. Moreover, many other measures must be executed before showering can be of any use. If visitors do not pass a hygiene lock or do not wear herd specific boots and clothing, a shower is redundant. As for the animal-free period, it is for instance not realistic to expect a veterinarian to only visit one farm a day. These measures (showering and an animal-free period) were not mentioned during the first study. Perhaps, they were not regarded as biosecurity measures or were considered too difficult to implement.

Participants of both parts of the first study were convinced that the application of biosecurity measures has advantages, which is in agreement with the research of Laanen et al. (2014). They generally situated these advantages in the improvement of herd health, which would benefit them financially. Such a positive opinion on a biosecurity measure correlates positively with the implementation of that measure (Valeeva et al., 2011). However, during the second study, five out of twenty biosecurity measures scored low (less than 0 on average on a Likert scale of -2 to +2) for all criteria judged (Figure 1). In both the focus group and in the survey, the disadvantages were emphasized. Members of the focus group discussion mentioned time and labor as disadvantages, while only half (48%) of the participants of the survey agreed with them. In the survey of Brennan en Christley (2013), similar results were found, while in the study of Laanen et al. (2014) and Jansen et al. (2010a) lack of time was the lowest judged reason for not using biosecurity tools. However, it can be questioned whether implementing biosecurity indeed takes more time or if it is only perceived as such. After all, many biosecurity measures include a structured, repetitive method, which is expected to gain time. Changing the modus operandi seems to be the challenge (Kristensen and Jakobsen, 2011), as farmers are inclined to carry on in the way they are used to (Casal et al., 2007).

The participants of the focus group indicated the need for more information in a simple, practical manner. This finding is in agreement with earlier research, which state insufficient, confusing or lack of herd-specific information as obstacles to implement biosecurity (Toma et al., 2013; Laanen et al., 2014; Sayers et al., 2014). The farmers of the present and other studies consider their herd veterinarian as the most reliable source of information on biosecurity (Brennan and Christley, 2013; Garforth et al., 2013). The veterinarian is the key actor to help farmers understand the logical reasoning behind proposed biosecurity measures, because the farmers know him/her and trust in his/her advice and capability (Racicot et al., 2012; Alarcon et al., 2014; Laanen et al., 2014).

During the first study, many possible reasons were mentioned, which could explain the line of thought of the participants of the second study. In the second study, the measure of keeping a closed farm (no purchase) could have been added; yet, it was chosen to assess two measures closely related, i.e. quarantine and same supplier. The farmers in the first study found it to be a measure of high importance but believed it to be hardly feasible. In addition, they mentioned quarantining purchased animals and found it to be an efficient measure. However, they believed a quarantine period of three weeks and fully physical separation hard to achieve. Their opinion concurs with the opinion of the participants of the second study, as they rated quarantine fairly efficient (fifth) but gave a lower score for feasibility (twelfth). The score for ROI (eleventh) was comparative to feasibility. Farmers of the first survey indeed questioned the cost of a stable that is used only a few times a year even if they agreed it has benefits. Buying animals from only one supplier was not thought of during the first survey.

The idea of herd-specific boots and clothing was not well-received in both studies. During the second study, the scores were in the middle of the ranking (ninth and tenth ranks) showing similarities to the results of the first study. Farmers in the first study were reluctant to ask visitors to wear herd-specific boots and clothing and claimed the visitors themselves were reluctant as well. They found the measure cumbersome and hard to execute consistently, as there are for example multiple entrances to the farm, even though all facilities were present. Perhaps the measure would have been judged more efficient in the second study, if practical problems concerning the proper execution would be solved. For example: a simple hygiene lock located at a critical juncture where all visitors should pass, could solve the issue of having multiple entrances. However, in the second study, a measure pertaining to this was judged even less feasible.

To understand why implementing biosecurity measures is beneficial, the farmer must first understand the risk of introduction and harm caused by both epidemic and endemic diseases. Evidence of the economic benefit of biosecurity measures is limited as quantitative data on costs and benefits are lacking.

In this respect, farmers may be reluctant to invest in biosecurity. In addition, cattle farmers are often not aware of the cost, neither of the loss of income caused by disease, which is even higher (Laanen et al., 2014). If farmers would be well-informed on both issues, they would be more willing to improve biosecurity on their farm.

Secondly, to obtain better results, a farmer should understand the rationale behind a biosecurity measure, as it is easier to exert and sustain a change in behavior if it is clear why that change has to be made (Wells et al., 2002). Other methods to influence the farmer's behavior are for instance the campaign described by Jansen et al. (2010b), in which a simple message conveyed with humor and free samples was meant to convince farmers to use milking gloves. However, it would be useful to understand the motivation and reasons behind the behavior of farmers, in which the second study of this paper might be of assistance. After all, it would be hard to convince farmers to change their behavior if they themselves were not convinced or had no intention to change (Kristensen and Enevoldsen, 2008; Brennan and Christley, 2013).

Therefore, in analogy with the pig and poultry sectors, cattle farmers could be provided with accessible and practical information through a biosecurity testing tool, which explains the concept of biosecurity and increases their awareness (www.BioCheck.UGent.be; (Laanen et al., 2010; Gelaude et al., 2014). It may also be useful to list the mechanisms of direct and indirect spread of disease, so farmers may gain insight into the effectiveness of biosecurity measures (Nöremark and Sternberg-Lewerin, 2014). Secondly, a preventive strategy could be developed in collaboration with the herd veterinarian who knows the herd structure and may inform on the critical points for that specific farm (Villarroel et al., 2007; Ellis-Iversen et al., 2010; Brennan and Christley, 2013; Garforth et al., 2013). The ultimate goal should be to consider biosecurity as a collective responsibility with farmers taking initiative to improve their biosecurity level and herd veterinarians as coaches, rather than an individual responsibility (Postma et al., 2017). Thirdly, to be able to influence the farmers' behavior, it must be understood first. It would be useful to understand motivational and behavioral factors of the farmers in deciding on issues related to biosecurity (Kristensen and Enevoldsen, 2008).

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Uit het verleden

ONGELIJKHEID BEGINT BIJ DE TREKOS EN HET PAARD

Ongelijkheid begon in de prehistorische samenlevingen met de beschikbaarheid van paarden en andere trek- en lastdieren. Die konden ploegen en karren trekken. Ze leverden bovendien mest, waardoor de opbrengst van de landbouwgronden aanzienlijk kon verhogen. Vlees, melk en huiden waren ook aardig meegenomen. Rij- en lastdieren maakten handel over grote afstand mogelijk, denk maar aan de Zijderoute. Handel is de beste manier om rijkdom te vergaren. Maar paarden (en kamelen) waren ook krachtige offensieve wapens. Zo ontstond er een bereden elite. Die kon in minder dan geen tijd grote gebieden veroveren. Denk aan de Hunnen en Djengis Khan. 'Ridder' betekent niet toevallig ruiter. En waarom de adel in Frankrijk zich liet aanspreken met 'chevalier' is al even duidelijk. Te paard kon je bovendien veroveringstochten organiseren die verder reikten dan het naburige dorp. In het prehistorische Amerika was de ongelijkheid minder groot dan in Eurazië. Daar had je paarden noch runderen. Inca's en aanverwante volkeren hadden geen karren of wagens. Ze beschikten immers niet over geschikte trekdieren.

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Luc Devriese

Feline arterial thromboembolism: prognostic factors and treatment

Feliene arteriële trombo-embolie: prognostische factoren en behandeling

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ABSTRACT

Feline arterial thromboembolism (ATE) is a complete or partial obstruction of a peripheral artery caused by a thrombus that was formed at a distant site. The most common underlying cause in cats is cardiomyopathy. Given the clinical presentation, often without preceding signs, an ATE event is considered one of the most distressing emergency conditions in feline practice. Often, these cats are euthanized at the time of diagnosis. However, recent scientific research has shown that a subpopulation of these patients may have long-term survival. In case of adequate treatment and follow-up, some cats survive over a year with a satisfying quality of life. Key points of ATE are the identification of specific prognostic factors in the individual patient in order to guide owner communication, the decision to treat or not to treat, individually adjusted patient management and regular monitoring, which are discussed in this article.

SAMENVATTING

Feliene arteriële trombo-embolie (ATE) wordt veroorzaakt door een volledige of gedeeltelijke obstructie van een perifere arterie ten gevolge van trombus die gevormd werd op een andere plaats; dit vaak ten gevolge van cardiomyopathie. Gezien de klinische presentatie, waaraan vaak geen symptomen vooraf gaan, wordt ATE beschouwd als een van de meest verontrustende, spoedeisende situaties in de praktijk. Een aanzienlijk deel van deze patiënten wordt geëuthanaseerd op het moment van diagnose. Recent wetenschappelijk onderzoek heeft echter aangetoond dat sommige van deze patiënten een goede langetermijnoverleving kunnen hebben. Deze katten kunnen soms tot langer dan één jaar overleven met een goede levenskwaliteit, mits een correcte behandeling en nauwkeurige opvolging. Essentieel bij deze aandoening is het herkennen van specifieke prognostische factoren om de communicatie met de eigenaar te sturen en zo in samenspraak de beslissing te nemen het dier te behandelen of te euthanaseren. Verder zijn een individueel aangepaste therapie en gerichte follow-up van de patiënt belangrijk.

INTRODUCTION

In feline practice, the occurrence of arterial thromboembolism (ATE) is one of the most distressing emergency situations for the cat, the client and the veterinarian. Currently, in first-line practice, these patients are commonly euthanized at the moment of diagnosis (Borgeat et al., 2014). Affected patients are usually presented with severe pain and associated vocalization, often combined with paralysis of the pelvic limbs and dyspnea or tachypnea. The most common form of ATE, the so-called "saddle thrombus", is caused by occlusion at the level of the distal aortic trifurcation. Diagnosis is primarily based on physical examination, with the typical clinical signs of pain, pulselessness, paralysis/paresis, pallor and poikilothermy, also referred to as the 5 P's. As an underlying cardiomyopathy is the most prevalent etiological cause, it is vital to recognize the presence of possible concomitant congestive heart failure (CHF), as this will influence both further medical management and prognosis. On auscultation, the presence of inspiratory crackles, a gallop rhythm or a murmur is highly suggestive of the presence of concomitant CHF. In general, the first

treatment step for these painful and often tachypneic/dyspneic cats consists of providing adequate pain relief medication and a stress-free environment with oxygen supplementation. Further investigations may be undertaken once the cat's pain, hypercoagulable state and possible CHF have been addressed via analgetic, antithrombotic and diuretic treatment. Although many of these cats are euthanized at the time of diagnosis, a correct identification of specific prognostic factors obtained from results of physical examination and further testing, enables the veterinarian to give the owner the best possible advice regarding further treatment, management and prognosis. The owner should be informed thoroughly about in hospital treatment and daily home medical care, the risk of ATE recurrence, presence of underlying cardiomyopathy, the need for monitoring, and associated costs. In this review, the pathophysiology of ATE is briefly explained to better understand clinical signs and diagnosis, with focus on prognostic factors aiding in the decision to treat, and different treatment options are discussed.

PATHOPHYSIOLOGY

Physiological thrombogenesis

In a healthy individual, thrombus formation is coordinated via interacting signalling pathways between platelets, coagulation factors and the endothelium, resulting in hemostasis without the risk of inappropriate thrombosis. Under physiological conditions, an intact endothelium normally produces antithrombotic factors, which help to maintain thromboresistance, whilst a damaged endothelial surface will trigger the coagulation cascade (Kapur et al., 2007). Furthermore, platelets will be activated via alternative pathways as well. The combination of these divergent pathways lead to a growing platelet plug with numerous platelets bridged together by fibrinogen. Initially, the thrombus is platelet-rich but quickly becomes fibrin-rich as the clot grows and matures (Helenski and Ross, 1985; Furie and Furie, 2008; Goggs and Poole, 2012).

Arterial thromboembolism

The thrombus becomes lamellated as it ages and superficial portions can break off, or more rarely, it dislodges in entirety, forming the emboli that travel to distant sites where their size eventually exceeds vessel diameter, resulting in infarction of an arterial bed (Collet, 1930). The thrombus causes a (partial) block of the obstructed artery and induces vasoconstriction of the collaterals, reducing perfusion and oxygenation of the tissue distal to the obstructed vessel. In cats with cardiomyopathy, the left auricular appendage is usually the site of thrombus formation. Certain arteries, e.g. the distal aorta, are more prone to obstruction, due to their anatomical conformation (Figure 1).

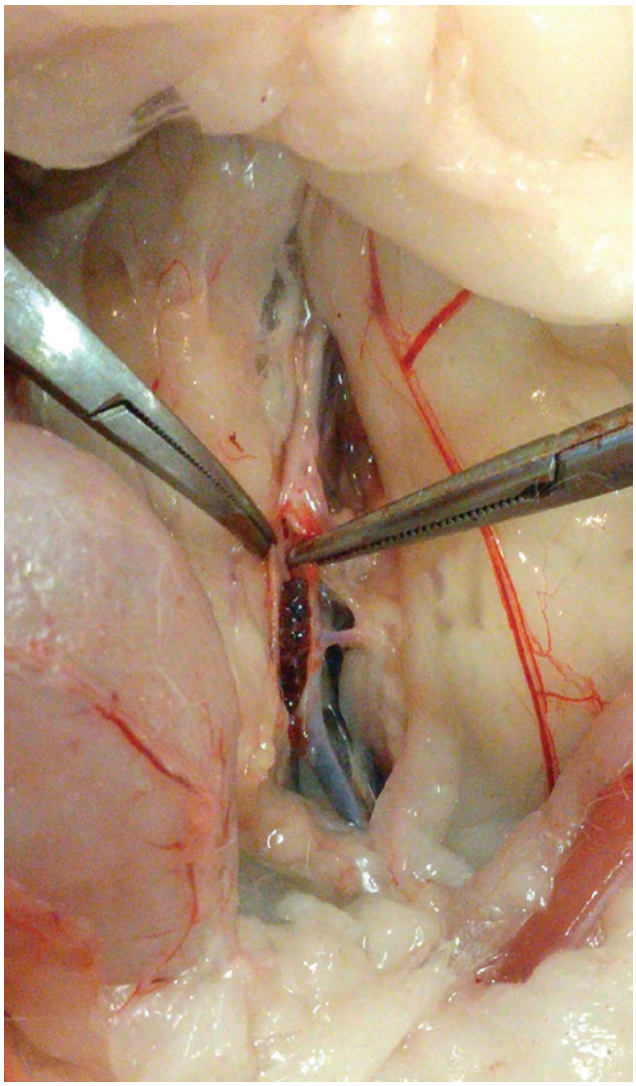

Figure 1. A thrombus obstructing the distal aorta at the level of the bifurcation (Photograph courtesy of P. Smets).

The most common underlying cause of thrombogenesis leading to an ATE event, is cardiac disease, and in the feline population, this is most commonly (in approximately 69% of the cases) cardiomyopathy (Smith et al., 2004). Feline cardiomyopathy predisposes to formation of intracavitary thrombi due to blood stasis, endothelial injury and a hypercoagulable state, fulfilling all components of the Virchow triad (Welles et al., 1994; Schober and Maerz, 2006; Stokol et al., 2008).

Blood stasis

Blood stasis is primarily seen in the left atrium, when it is dilated secondary to the underlying cardiac disease. Low blood flow velocities have been linked to spontaneous echo contrast or 'smoke' in the left atrium and left auricular appendage, which can easily be detected using echocardiography (Figure 2).

Endothelial function

Secondary to left atrium enlargement, increased intra-atrial pressure and stretch have been associated with endothelial injury, exposing subendothelial collagen or causing endothelial dysfunction, which facilitates platelet adhesion with subsequent activation and aggregation (Laste and Harpster, 1995; Smith et al., 2003; Furie and Furie, 2008).

Platelet function

The presence of a hypercoagulable state in cats is controversial, but the clinical occurrence of thrombosis has been associated with platelet hypersensitivity, increased procoagulantia and decreased antithrombin levels (Jandrey et al., 2008; Brazzell and Borjesson, 2007). However, neither left atrial size nor CHF were associated with hypercoagulability in a study by Stokol et al. (2008). This suggests that a subset of cats with cardiomyopathy may be hypercoagulable and develop ATE irrespective of atrial enlargement (Welles et al., 1994; Stokol et al., 2008).

Having discussed Virchow's triad and its different components in association with the pathogenesis of arterial thromboembolism, it can be concluded that an ATE event is a multifactorial process, with the most important factor being blood flow stasis associated with a dilated left atrium; other contributing factors, such as endothelial damage or hypercoagulability, may be involved to a lesser extent (Fuentes, 2012).

PREVALENCE AND SIGNALMENT

In several studies, divergent prevalence numbers have been shown, ranging from 0.6% in referral populations to 0.3% in general practice (Smith et al., 2003; Borgeat et al., 2014). In different studies, an ATE prevalence has been reported from 12 up to 21% in cats that have been diagnosed with HCM (Rush et al., 2000). Tomcats are overrepresented. This might reflect the male predisposition to cardiomyopathy, which is hence likely to be gender biased. There is no real breed predisposition, purebred cats are affected as well as domestic short- and longhair cats (Baty et al., 2001; Ferasin et al., 2003). Age at presentation with ATE ranges from one to twenty years, but the median age is eight to nine years (Laste and Harpster, 1995).

HISTORY AND CLINICAL SIGNS

Affected patients are usually presented with severe pain and associated vocalization, often combined with paralysis of the pelvic limbs and dyspnea or tachypnea. Because the trifurcation of the distal aorta is the most prevalent site of obstruction, an ATE event often leads to paresis or paralysis of the pelvic limbs due to ischemic neuromyopathy, although one leg might be more affected than the other. Infarction of one thoracic limb is the second most common clinical presentation of ATE in cats, with reported frequencies of 12-19% (Hogan and Brainard, 2015). An obstruction of the brachial artery will lead to paresis or paralysis of the affected thoracic leg. Tetraparesis has been reported as well (Bowles et al., 2010). Clinical signs might be different with embolization sites other than the distal aorta, the communal iliacal arteries, or the brachial artery and are dependent on the secondary affected and ischemic organs. The majority of cats with an ATE event that are presented in practice have no known history of cardiomyopathy. Severe pain and often paralysis of the pelvic limbs are often the first clinical signs noticed. Its dramatic presentation and the lack of preceding clinical signs make ATE one of the most distressing conditions encountered in feline practice (Fuentes, 2012).

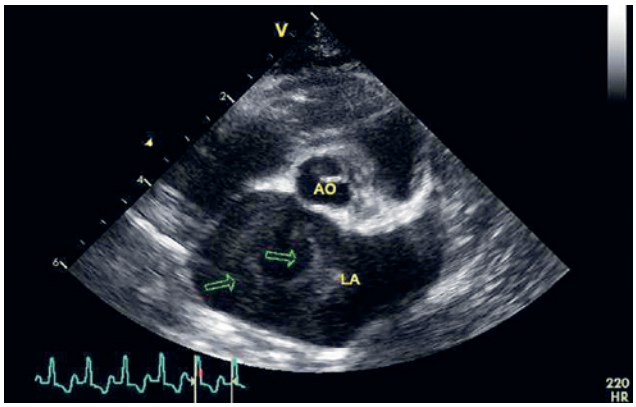

Figure 2. Spontaneous echocardiographic contrast, also known as 'smoke', can be distinguished within the enlarged left atrium (arrows) (LA: left atrium; Ao: aorta) (Photograph courtesy of P. Smets).

Figure 3. Abnormal stance (knuckling of the metatarsus) with paralysis of the right hind limb due to ATE (Photograph courtesy of P. Smets).

PHYSICAL EXAMINATION

Besides pain and distress often in combination with vocalization, dyspnea and tachypnea, the clinical signs are associated with the affected site of ob-

struction. As the trifurcation of the distal aorta is the most affected site, representing 71% of the cases in one large study, paresis or paralysis of either or both hind legs due to a so called "saddle thrombus" are most commonly seen. Studies have shown that only one limb may be affected in up to 26% of cats with ATE (Smith et al., 2003) (Figure 3). The diagnosis of saddle thrombus can be made from the physical examination alone, based on the following signs: pain, paralysis/paresis of the hind limbs, pulselessness, pallor and poikilothermy, also referred to as the 5 P's. The combination of pelvic limb lower motor neuron signs with absent femoral pulses and cold extremities is pathognomonic for ATE and allows differentiation with a neurological cause. Pulselessness might be hard to differentiate in the front legs, but in addition to cold extremities, the foot pads of the affected limb(s) are often pale or cyanotic (Figure 4). If the embolized thrombus is small, rapidly lyzed or collateral function is quickly re-established, motor function may be present, and foot pads may be well-perfused by the time the cat is presented. The last P, namely the poikilothermy, refers to the fact that the majority (up to 72%) of ATE cats are presented with a reduced rectal temperature, which is considered to be a poor prognostic parameter when it is below 37.2°C (Smith et al., 2003; Borgeat et al., 2014). The rectal tone and urinary bladder function are typically maintained.

Furthermore, signs of CHF, e.g. dyspnea and tachypnea, might be present. It is vital to recognize the presence of possible concomitant heart failure, as this will influence the further medical treatment, management and prognosis. Congestive heart failure has been reported in 44% to 66% of cases (Smith and Tobias, 2004). An increased respiration rate should not automatically be interpreted as diagnostic of CHF, since the animal may be tachypneic only due to severe pain. Re-assessment after adequate analgesia is necessary in this case. On the contrary, the presence of crackles, a murmur, gallop sound or arrhythmia detected during thoracic auscultation are suggestive for CHF and possible pulmonary edema. However, in 40% of the cats presented with ATE, auscultation is unremarkable, despite the fact that myocardial disease is the underlying cause in the vast majority of cases (Laste and Harpster 1995). Therefore, underlying heart disease based on a normal auscultation cannot be ruled out (Wagner et al., 2010).

DIFFERENTIAL DIAGNOSIS AND DIAGNOSTIC TOOLS

Differential causes of acute posterior paresis include: trauma, intervertebral disc extrusion, spinal lymphoma and other neoplasias, fibracartilaginous embolism-induced infarction (Volk et al., 2011). Acute forelimb paresis can be caused by trauma, foreign body, brachial plexus avulsion.

It is important to emphasize that administration of adequate pain medication (usually opioids), oxygen supplementation in case of dyspnea and reducing stress to an essential minimum are vital parts of the first crucial management step and precedes any further diagnostic tests.

Thoracic radiographs

Thoracic radiographs are currently the most reliable diagnostic test in order to confirm or exclude the presence of CHF, especially if thoracic auscultation was unremarkable; moreover, they rule out other possible, pulmonary pathologies, which might cause tachypnea or dyspnea. However, in one study, about 11% of cats with thromboembolism had normal cardiac silhouettes (Laste and Harpster, 1995). In cats with respiratory distress, thoracic radiographs should be postponed until the patient is stabilized, as manipulation might cause further (sudden) clinical deterioration.

Echocardiography

Once the patient is clinically stable, an echocardiographic examination is advised in order to diagnose a possible underlying heart disease. The vast majority of these patients have a dilated left atrium, possibly in combination with spontaneous echogenic contrast or 'smoke' visible in the left atrial lumen (Figure 2). 'Smoke' indicates an increased risk of embolic stroke in human patients, and is believed to be associated with an increased risk of ATE in cats (Schober and Maerz, 2006). However, a small subset of cats presented with ATE do not have cardiac abnormalities on echocardiography.

Figure 4. Cyanotic left hind limb footpad and the pale pink right hind limb footpad in this cat with asymmetric ATE of the distal aorta (Photograph courtesy of P. Smets).

Although the diagnosis of ATE in cats with pelvic limb paresis/paralysis is usually based on the distinctive clinical findings rather than imaging, it is commonly possible to identify the thrombus in the terminal aorta using ultrasound imaging. However, the absence of a visible thrombus does not rule out ATE. Where no underlying cardiac disease is identified, further local imaging of the occluded artery is more justifiable (Fuentes, 2012).

Additional tests

Biochemistry tests

Biochemistry tests may show a variety of abnormalities, e.g. azotemia, hyperglycemia and phosphatemia. Azotemia can be either prerenal secondary due to reduced renal perfusion or renal due to thromboembolism of a renal artery. Hyperglycemia is often stress-related. Furthermore, serum creatine kinase is often severely increased due to muscle ischemia. Furthermore, alanine aminotransferase values are often elevated (Smith et al., 2003). Potassium values are variable and cats may actually be hypokalemic on presentation, but may develop hyperkalemia once perfusion has been restored. Finally, hypocalcemia and hypernatremia have also been reported. In older cats, total thyroxine levels should be measured as hyperthyroid cats may be at an increased risk of ATE irrespective of any cardiac changes (Laste and Harpster, 1995). Coagulation tests are frequently normal, although D-dimers may be elevated (Bedard et al., 2007; Fuentes, 2012).

Blood pressure measurement

Doppler blood pressure assessment may be used to confirm the lack of pulse in the affected limb.

Electrocardiography

Electrocardiography was shown to be informative in a large retrospective study of cats presenting with ATE, with 85% of affected cats having ECG changes in heart rate or rhythm. The most common ECG abnormalities included a left ventricular enlargement pattern, with large R-waves in leads I and aVL (39%), sinus tachycardia (28%), ventricular premature beats (19%), supraventricular premature beats (19%), prolongation of the QRS interval (16%) and a left atrial enlargement pattern (16%), with a broad, bifid P wave in lead II (Laste and Harpster, 1995).

DECISION TO TREAT AND PROGNOSIS

Despite the fact that these patients are often presented with acute limb paralysis in severe pain and distress, which is often perceived by both the owners and veterinary practitioner as inevitably disastrous, a significant subset of cats may recover and lead good-quality lives. The general prognosis is poor with a survival rate of 33% to 39%, but several cats may display positive prognostic factors indicative of higher survival rates and better mid- to long-term prognosis. Those positive prognostic factors are the absence of CHF, rectal temperature at the moment of presentation >37.2°C, (partial) presence of motor function, and the site of thromboembolism and associated affected limbs or other organs (Schoeman, 1999; Borgeat et al., 2014). Although many of these cats are euthanized upon diagnosis, a correct identification of specific prognostic factors obtained from results of physical examination and further testing, enables the veterinarian to give the owner the best possible advice regarding further treatment, management and prognosis. Despite the generally bad prognosis, a study by Smith et al. (2003) showed that rates of survival to discharge improved over a ten-year period, with 73% of cats surviving to discharge in the last year of study. As many as 70–80% of cats with a single limb affected survived to discharge, with up to 90% survival rates in cats presenting with some motor function. Hypothermia appeared to be one of the most reliable markers of reduced initial survival in this study, with <50% chance of survival to discharge in cats with a rectal temperature <37.2°C on presentation.

Pain is severe in the initial 24 hours, but decreases substantially after the first 48 hours. The cranial tibial and gastrocnemius muscles may become firm within ten to twelve hours post-embolization due to ischemic myopathy, and ischemic nerve damage may result in ‘dropped hocks’ as well as loss of distal sensation. The muscles become softer 24 to 72 hours later. However, in some cats, the degree of firmness increases over the first few days, suggesting poorer perfusion. Perfusion and femoral pulses frequently return within three to five days, although in case of severe tissue ischemia, there is a risk of skin and muscle necrosis (Figure 5). This usually manifests within the first two weeks and may lead to loss of toes or decision for limb amputation in severe cases (Smith et al., 2003). Although not well-quantified, many cats may regain

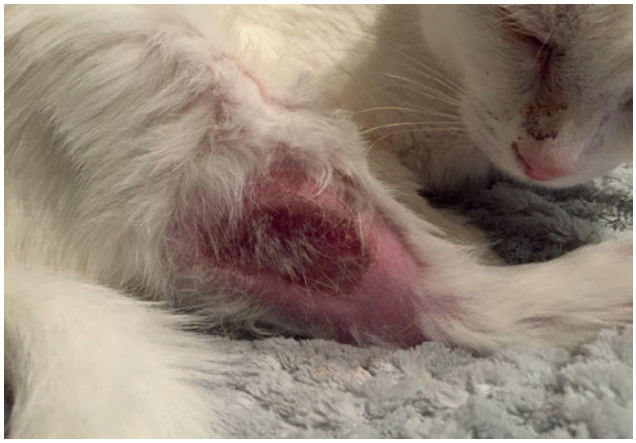

Figure 5. Skin necrosis of the right hind limb, secondary to an ATE event (Photograph courtesy of L. Locquet).

some to all motor function of the pelvic limbs within four to six weeks from the initial event either due to re-establishment of the collateral vascular network, intrinsic dissolution of the embolus, or recanalization of the obstructed aorta (Smith et al., 2003).

Owners should be aware that their cat might not survive to discharge or might develop a following episode of ATE in the (nearby) future. However, especially cats without CHF and with only one affected limb, remaining motor function and a rectal temperature above 37.2° have a substantially better prognosis and may regain partial or complete normal function. Reported ATE recurrence rates range from 24-75%, but because cardiomyopathy is the most prevalent etiological cause, the major cause of death in this subgroup is actually CHF, and not a recurrent episode of ATE (Rush et al., 2000; Lunsford and Mackin, 2007; Payne et al., 2015). Reports of average long-term survival vary between 51 and 350 days (Atkins et al.,1992; Schoeman, 1999).

TREATMENT OF ATE

Thrombolytic therapies

Several approaches have been tested in order to remove the obstructing thrombus, but the small size of cats limits minimal invasive embolectomy techniques. Moreover, anesthetic and surgical complications cause high mortality rates. Therefore, this type of treatment is currently contraindicated (Reimer et al., 2006). Medical thrombolytic therapy with streptokinase and tissue plasminogen activator (t-PA) have both been reported in the cat and dissolution of the thrombus has been documented up to 18 hours after initial clinical signs (Moore et al., 2000; Welch et al., 2010). However, sudden reperfusion of ischemic tissues flushing ischemic metabolites, such as potassium and free oxygen species into the bloodstream, leading to reperfusion injury, a life-threatening complication causing arrhythmias, renal dysfunction and acid-base disturbances. This has been reported in 40% to 70% of cats receiving thrombolytic therapy and represents the most common cause of death, with survival rates ranging from 0% to 43% (Lunsford and Mackin, 2007; Welch et al., 2010). Given these possible lethal complications, thrombolytic therapy should not be administered without thorough consideration of the benefit-to-risk ratio, which should be evaluated for each individual case and its clinical presentation. If available, thrombolytic therapy should be considered in rare cases of cerebral, splanchic or renal infarction, while a cat with a unilateral limb infarction is more likely to achieve complete recovery with conservative therapy (Reimer et al., 2006). The high costs also limit medical thrombolytic therapy in veterinary medicine.

Since thrombolytic therapy is often not possible or even contraindicated, the mainstay of treatment is prevention of continued thrombus formation. The two major categories of antithrombotic drugs are antiplatelet agents and anticoagulants (Fuentes, 2012; Hogan and Brainard, 2015). Doses for these drugs can be found in Table 1.

Antiplatelet agents

Antiplatelet agents are currently the mainstay of treatment for feline ATE, because they may improve collateral flow by reducing the amount of vasoactive substances that are released by the platelets (Schaub et al., 1982; Arrebola et al., 2004). Some veterinary cardiologists recommend a combination of two antiplatelet agents (clopidogrel + aspirin) in cats, although there are no studies in cats available about this approach (Fuentes, 2012). This approach is typically limited to those patients that appear to be at particularly increased risk of ATE, i.e. those cats, which continue to have spontaneous echogenic contrast or develop a left atrial thrombus in spite of already receiving therapy (Tan et al., 2010).

Aspirin

Aspirin irreversibly inhibits the production of thromboxane A2 (TXA2), a potent pro-aggregating and vasoconstrictive molecule. This effect lasts for the lifetime of the platelet. Treating feline patients with aspirin prevent vasoconstriction of collateral circulation. However, cats are still at risk to develop ATE despite treatment, as other TXA2-independent pathways may still result in platelet aggregation (Lunsford and Mackin, 2007). Smith et al. (2003) have shown that the incidence of ATE with low-dose aspirin (5mg/cat q72h) compared to high dose aspirin (40mg/cat q72h), was the same. When applying the latter dose, adverse effects are typically gastrointestinal, including anorexia and vomiting secondary to gastric ulceration, and have been reported in up to 22% of treated cats. Aspirin has been used for the prevention of recurrence of ATE in cats that already had an episode of ATE (secondary prevention) with recurrence rates ranging from 17% to 75%, and median survival times from 117 to 192 days. However, the recent prospective FATCAT study, evaluating secondary prevention of ATE in cats, has shown aspirin to be inferior to clopidogrel for secondary prevention (Hogan et al.,2015). If monitoring of aspirin therapy is desired, platelet aggregation should be performed before and after at least two weeks of therapy to determine the degree of platelet inhibition. However, it should be mentioned that only a minority of institutions offer this aforementioned test.

Clopidogrel

Clopidogrel is a specific and irreversible ADP receptor antagonist, as well as a direct platelet antagonist, inhibiting both primary and secondary platelet aggregation. Additionally, clopidogrel also has been

shown to impair the platelet release reaction and to have vasomodulating effects, maintaining collateral circulation parallel to the occluded artery. Adverse effects are rare, although there are empirical reports of sporadic hypersalivation due to the bitter taste of the drug, vomiting and icterus (Hogan et al., 2015). Clopidogrel has been shown to be superior to aspirin for secondary prevention, with a lower ATE recurrence rate of 49% (versus 75%) and a one-year recurrence rate of 36% (versus 64%). Additionally, clopidogrel has been associated with longer median time to event (443 days) than aspirin (192 days) (Hogan et al., 2015). As with aspirin, monitoring of clopidogrel therapy is rarely performed in veterinary medicine but ADP-induced platelet aggregation is considered the gold standard.

Anticoagulants

Anticoagulants inhibit the coagulation cascade by interfering with formation of one or more active coagulation factors. In feline medicine, they are now less commonly used than anti-platelet agents, but the new classes of drugs, such as factor Xa-inhibitors may hold promise for future use in cats.

Unfractionated heparin

Heparin inhibits the coagulation process by counteracting thrombin and activate factors XII, XI, I and IX (Lunford and Mackin, 2007). Heparin is administered SC, but if the cat is in shock, the first dose may also be administered IV. Heparin is often used early in the course of feline ATE to reduce extension of the existing thrombus, and risk of hemorrhages appears to be small. Serial measurement of aPTT has historically been used to monitor effect, with desired aPTT 1.5-2.0 times above baseline. However, in one study, it has been shown that aPTT does not correlate well with plasma heparin levels and anti-Xa monitoring may be a better approach (Smith et al., 2004).

Low molecular weight heparins

Given its longer half-life and more predictable dose response, low molecular weight heparin (LMWH) has largely superseded unfractioned heparin in the prevention of human deep vein thrombosis, enabling the practitioner to adjust the dose more accurately according to the body weight without the need to monitor the coagulation effects (Lunford and Mackin, 2007). Due to the pharmacokinetic profile and positive clinical studies in humans, there has been great interest in the LMWHs for the prevention of ATE in cats (Smith et al., 2004; Vargo et al., 2009). Alwood et al. (2007) compared enoxaparin, dalteparin and unfractrioned heparin in healthy cats. Only unfractionated heparin showed adequate anti-Xa activity. However, the anti-Xa activity does not always correlate with clinical antithrombotic effects. There is one retrospective study,

Figure 6. Skin necrosis in the same cat, healing by secondary intention, secondary to an ATE event (Photograph courtesy of L. Locquet).

in which dalteparin was compared to warfarin, and no significant difference in ATE recurrence rate (43% versus 24% respectively) or median survival time (255 days versus 69 days) was demonstrated (Defranscesco et al., 2003). At this timepoint, for the use in the acute in-hospital treatment, there is no clear benefit of LMWH over unfractionated heparin, whereas the cost of the former is much higher.

Warfarin

Warfarin blocks the effects of vitamin K, necessary for coagulation factors II, VII, IX and X to be activated. However, it is highly protein-bound, has numerous possible interactions with other medications, and has unpredictable pharmacodynamics and pharmacokinetics, with the latter being influenced by both disease state and dietary components. This leads to difficulties associated with its use, safety and effect (Lunsford and Mackin, 2007). Given the unpredictable effect, feline patients exhibit a wide and variable inter-individual and intra-individual anticoagulant response to warfarin, resulting in poor results in studies on warfarin use in cats, with cats with continued thrombogenesis and cats with severe hemorrhages. ATE recurrence rates for cats receiving warfarin range from 24% to 53% with estimated mean survival times of 255 days (Defranscesco et al., 2003).

Conclusively, necessary regular monitoring of prothrombin time and corresponding blood sampling in order to assess the effect of warfarin, compromises the life quality of these patients. Although the superiority of warfarin has been demonstrated for the prevention of ATE in humans, the issues with variability in clinical response, requirement for frequent monitoring and bleeding complications have resulted in a rare use of warfarin for thromboprophylaxis in cats.

Table 1. Possible medical management options in the initial and further treatment of ATE (adapted from Luis-Fuentes, 2012).

	Drugs	Dose	Advantages	Disadvantages	Comments
Analgesics	Methadone	0.2-0.4mg/kg slow IV q4-6h	Potent analgesia Vomiting is rare		Mu agonist, titrate to effect
	Fentanyl	3µg/kg slow IV, followed by 2-5 µg/kg/h CRI	Potent analgesia	Mu agonist, titrate to effect	
	Buprenorphine	0.02mg/kg IV or IM q6h	Widely available	Not sufficiently potent for severe pain	Partial opioid agonist
CHF treatment	Furosemide	1-2mg/kg q1-8h IV	Rapid diuresis	May cause azotaemia and hypokalemia	Confirm tachypnea is due to pulmonary oedema with thoracic X-ray
	Pimobendan	0.625-1.25 mg/cat q12h PO	May improve hypotension	Oral medications difficult with severe respiratory distress	May worsen outflow tract obstruction, so do not use if murmur is present
Anticoagulants	Unfractionated heparin	100-250 U/kg IV, 50-250 U/kg SC q6h	Inexpensive May reduce expansion or xtension of thrombusEffect can be monitored by prothrombin time	High doses result in a more rapid onset of effect compared with lower doses. Risk of haemorrhage appears to be small	Do not use IM- risk of bleeding
	Enoxaparin	1mg/kg q8-12h SC	Reduced risk of hemorrhage?	Expensive	Effective dose and dosing interval unkown
	Dalteparin	100 IU/kg q8-12h SC	Reduced risk of hemorrhage?	Expensive	Effective dose and dosing interval unkown
	Warfarin	0.5mg/cat q24h PO	Inexpensive Effect can be monitored with international normalized ratio	Unpredictable pharmacokinetics High risk of haemorrhage Requires frequent blood sampling to monitor effects	DO NOT USE
Antiplatelets	Aspirin	75mg/cat q72h PO (high dose) or 5mg/cat q72h PO (low dose)	Inexpensive Mostly well-tolerated Risk of hemorrhage low	Inferior to clopidogrel for secondary prevention of ATE	Low dose may also be given q48h
	Clopidogrel	Loading dose of 75mg Chronic: 18.75mg/cat q24h PO	Mostly well-tolerated May have additive effect with aspirin Superior to aspirin for secondary prevention of ATE Risk of hemorrhage low	Bitter taste, may cause salivation	

Newer anticoagulants

Recently, in human medicine, a wide variety of new anticoagulant drugs have been developed to prevent deep venous thrombosis, pulmonary embolism and ATE. Examples of these newly developed anticoagulants are abciximab (a glycoprotein IIb/IIIa antagonist), dabigatran (a direct thrombin inhibitor) and factor Xa-inhibitors, e.g. fondaparinux, rivaroxaban and apixaban. These drugs have been studied thoroughly within human medicine and were designed with excellent efficacy. They exhibit relatively low bleeding risks and generally do not require clinical monitoring. Studies regarding the use of these medications within veterinary medicine are currently lacking. It is expected that factor Xa-inhibitors will likely have a major impact on clinical prophylaxis or ATE in cats. Given the aforementioned characteristics, it would dramatically improve the clinical management of cats at risk of ATE, as current prophylactic protocols are still often based on theoretic effects and benefits, assumed bleeding risk and clinical experience (Hogan, 2017).

General treatment approach

Currently, a considerable part of these patients are euthanized upon diagnosis, given the sudden onset of devastating clinical signs, often without preceding warning. Given the very painful nature of this condition and due to welfare issues, euthanasia should be discussed and considered indeed, in case no adequate pain relief medication can be provided. Furthermore, failure to receive aspirin, clopidogrel or both is an independent predictor of euthanasia or death within seven days (Borgeat et al., 2014). However, if adequately treated, the majority of these patients are fairly stable within 48-72 hours. Aforementioned prognostic factors, i.e. the presence of CHF, bilateral hind limb paralysis versus only one limb affected, rectal temperature, etc., enable the clinician to have a more substantiated discussion regarding prognosis and expected success rate. Therefore, veterinarians and owners should consider therapy for at least the first 72 hours and not make a decision for immediate euthanasia (Hogan et al., 2015).

Therapy and management of a cardiogenic ATE (CATE) event can be divided in an acute and long-term component. The management of acute CATE includes: 1. provide pain management, 2. induction of a hypocoagulable state to reduce continued thrombus formation, 3. improve blood flow to the infarcted arterial bed, 4. treat concurrent CHF if present, 5. provide supportive care.

This approach does not require extensive financial resources, nor does it necessitate a prolonged hospital stay.

First 60 minutes

Even before having a thorough discussion with the owners regarding further treatment possibilities, analgesia has to be administered (Figure 7). Opioid analgetics, e.g. methadone, buprenorphine or fentanyl, should be administered at adequate doses and titrated to effect. Furthermore, in case of respiratory distress, oxygen supplementation should be provided without distressing the patient any further. Inspiratory crackles and a gallop rhythm are highly suggestive for the presence of concomitant CHF. If severe pulmonary edema secondary to CHF, is expected, an intravenous/intramuscular bolus of furosemide should be administered at 1-2mg/kg and repeated to effect. The bolus can be repeated hourly or even more frequently, depending on the severity of the respiratory distress and clinical response. Once the cat has been clinically stabilized, thoracic radiographs should be taken in order to confirm the presence of suspected CHF (Fuentes, 2012).

The next priority is to start antithrombotic treatment in order to induce a hypocoagulable state. Given the acute onset of clinical signs, the embolus has often broken off only recently and hence has a 'fresh' surface, which activates and stimulates the coagulation system, resulting in additional thrombus formation and vasoconstriction of the collateral circulation. The basic principle of this antithrombotic treatment is to avoid further extension of the existing thrombus, prevent additional thrombus formation and shift the intrinsic thrombosis/thrombolysis equilibrium toward thrombolysis. Hence, it is important to realize that the main goal is not to lyze the already developed thrombus (Hogan, 2017).

The value of unfractionated heparin or LMWHs for this use has been shown to be quite variable in cats. The recommended dosing regimen for unfractionated heparin is 250 to 375 IU/kg intravenously initially, followed by 150 to 250 IU/kg subcutaneously every six to eight hours. Heparin should not be administered intramuscularly because of the risk of injection site hemorrhage. Obtaining a coagulation panel that includes platelet count, PT and aPTT before unfractionated heparin administration is ideal so drug effect can be documented by repetitive re-evaluation of these aforementioned values during heparin therapy. Although not supported by clinical data, prolongation of the aPTT of 1.5 to 2.0 times the baseline value is considered an adequate drug response (Lunsford and Mackin, 2007). As unfractionated heparin is relatively inexpensive and readily available, it is a good choice for acute therapy.

Compared to unfractionated heparin, low molecular weight heparins have similar hemostatic effects; however, the costs are considerably higher. The current recommended protocols for dalteparin in cats

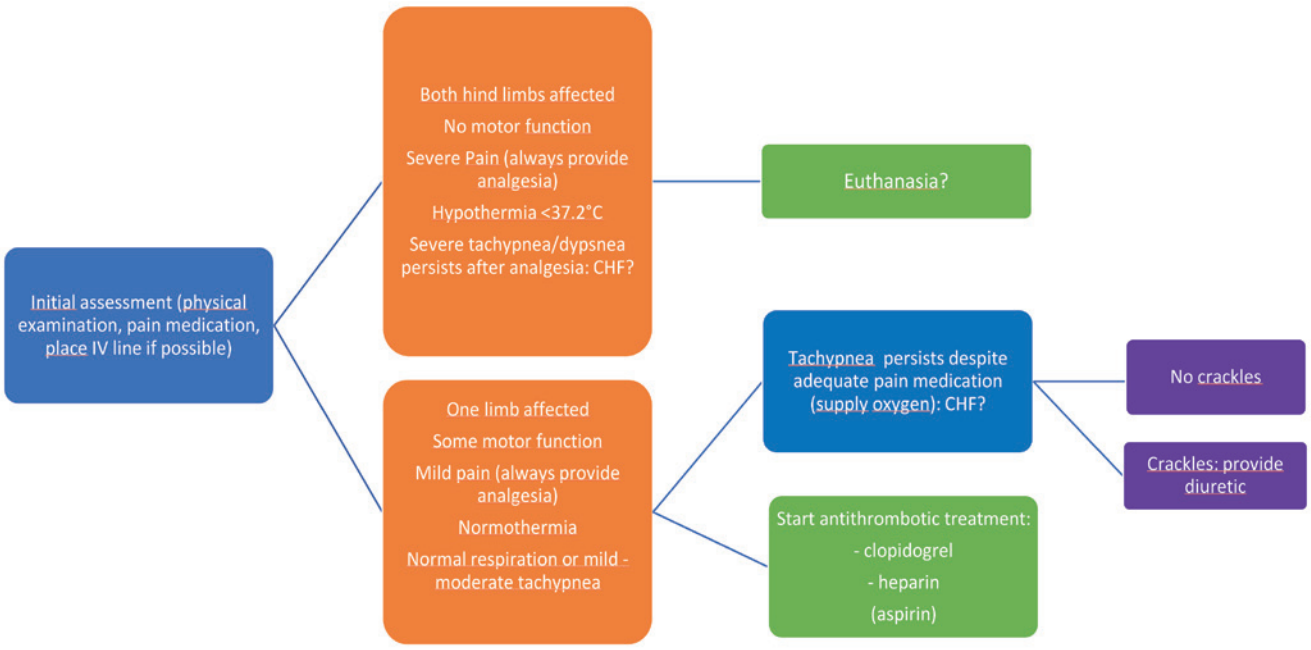

Figure 7. Approach to the cat with ATE: first hour (adapted from Luis-Fuentes, 2012).

are 100 IU/kg subcutaneously every 24 to 12 hours and for enoxaparin 1.5mg/kg SC every 24 to 12 hours, respectively. In a recent pilot study, it has been demonstrated that enoxaparin, when administered at a dosage of 1 mg/kg SC q12h already produced an antithrombotic effect in a venous stasis model in clinically healthy cats (Van De Wiele et al., 2010). Although they have a beneficial pharmacokinetic profile, allowing them to be administered twice daily, this effect is minimal in the acute management of ATE, so the higher cost of the LMWH may not be justified.

Increasing perfusion to the infarcted region can be attempted by increasing blood flow through the collateral network using antiplatelet drugs, which may be helpful by impairing platelet activation, and hence the vasoactive substances released from platelets. Either aspirin or clopidogrel can be used. Studies have shown that aspirin reduces the amount of released thromboxane A2, which results in an improvement of the collateral flow (Schaub et al., 1982). However, in these studies, very high aspirin doses were used (150mg/kg), which might cause toxic adverse effects. Lower doses have not been evaluated with regard to the effect on the collateral network. Similar results have been noted after clopidogrel administration at a dose of 75mg per cat, due to inhibition of platelet activation and secondary reduction of serotonin release.

An alternative approach is to use oral platelet inhibitors such as aspirin or clopidogrel from the start, and not include heparin at all. Some clinicians use both aspirin and clopidogrel together as early as possible (Fuentes, 2012).

Direct-acting arterial vasodilators such as acepromazine have not been shown to have an effect on the collateral network, and are contraindicated as hypotension may result in further reduced perfusion.

First 24 hours

Further tests and investigations may be undertaken once the cat's pain, ATE and possible CHF have been addressed. Renal values and electrolytes, especially potassium, should be evaluated and an echocardiographic examination should be performed in order to identify possible underlying cardiomyopathy.

Intravenous fluid therapy may assist the treatment of azotemia and the removal of metabolic toxins. However, as a substantial proportion of the patients suffers from CHF, it is recommended to cautiously use parenteral fluid therapy only in cases that would benefit from its use, which is a minority (Fuentes, 2012). Vigilant monitoring of respiratory rate, effort and auscultation for the development of a gallop rhythm is particularly important in these patients. Furthermore, in these particular cases, the diuretic dose should be reduced and other approaches to stimulate the heart function should be assessed.

Given its positive inotropic and lusitropic effect, pimobendan can be administered, although pharmocokinetic and dynamic studies of pimobendan in cats are scarce at the moment and its use is currently not authorized in cats (MacGregor et al., 2011). Pimobendan is not a standard part of the treatment strategy in CATE. Moreover, it may be contraindicated if systolic anterior motion of the mitral valve causing dynamic left ventricular outflow tract obstruction has been

diagnosed based on the echocardiographic examination. In this case, it may lead to hypotension (Gordon et al., 2012; Gordon and Côté, 2015).

Furthermore, an intensive care setting is essential in the further treatment of ATE cats in order to ensure that these patients are comfortable and early signs of possible complications are detected. Despite the cats being monitored regularly, manipulation should be kept to an essential minimum to prevent any further distress.

24-48 hours post-ATE

Further investigations/monitoring

Pain usually decreases and resolves over the first 24-36 hours, with an easily identified improvement, both in general as of the warmth, pulse quality and motor function of the affected limb(s) in particular (Hogan, 2017). Even without specific thrombolytic measures, the pulse strength will often improve within four to five days. This reflects an improved perfusion of the affected limb(s). Severe ischemic nerve damage may take weeks to improve.

In this particular phase, identifying early warning signs of possible complications or adverse effects is vital, although often challenging. The latter may include CHF, reperfusion injury, azotemia and local consequences of ischemic tissue necrosis. Reperfusion of ischemic tissue may cause fluctuating serum potassium levels, acid-base balance disturbances and elevated reactive oxygen species concentrations, which might be life-threatening. This may cause the patient to deteriorate dramatically and often very sudden, whilst often making apparent good progress before the reperfusion injury (Fuentes, 2012). In patients with ATE, only partially obstructing the affected blood vessel, reperfusion injuries are often less severe. Cardiac and neuromuscular cells are most sensitive for hyperkalemia, resulting in generalized lethargy and bradycardia, possibly evolving towards potential lethal atrial standstill and a comatose state. These can be confirmed with blood tests and electrocardiogram. Hyperkalemia should be treated accordingly with adjusted fluid therapy, glucose, (humilin) insulin, and/ or calcium bicarbonate, dependent on the exact elevated potassium levels and its availability.

Management

Antithrombotic treatment with clopidogrel, possibly in combination with aspirin, should be continued (Hogan et al., 2015). Despite the fact that most patients will be comfortable after 24 hours, the analgetic treatment should be continued (Table 1). Warm ambient temperatures may stimulate circulation and counteract muscle contraction. Application of heat pads or other external heat sources is contraindicated because of the risk for thermal injury to the infarcted tissues. Furthermore, nutritional support is a critically important aspect that may be overlooked. If the cat is not eating or the caloric intake is inadequate, administration of mirtazapine, an appetite stimulant, or assisted enteral feeding should be considered (Hogan, 2017). Self-mutilation of devitalized limbs occurs in a subset of patients and is characterized by excessive licking or chewing of the toes or hock. Application of a loose fitting bandage barrier is usually effective. Conclusively, it is important to manually void the urinary bladder if necessary, and maintain a clean and comfortable bedding.

>48 hours post-ATE

The vast majority of the cats are more comfortable and happier at home after the initial 72 hours post-ATE. Oral analgetics, e.g. buprenorphine, should be continued at home if there would be still any discomfort present at the time of discharge. The aspirin and clopidogrel can be continued for longer term prophylaxis (Hogan, 2017). Stool softeners, such as lactulose, can be administrated in case of hard stools or constipation. The owner should be informed thoroughly regarding the cat's need for relatively intensive daily home medical care, risk of ATE recurrence, prognosis of the underlying cardiomyopathy and necessity of monitoring the resting respiratory rate, as well as expected costs of treatment and follow-up. Detailed instructions should be given to the owner regarding the physiotherapy and early identification of possible chronic complications from aortic infarction, e.g. self-mutilation, limb necrosis requiring amputation and limb contracture (Fuentes, 2012) (Figures 5 and 6).

Rehabilitation therapy may be instituted after the first two days of ATE and includes gentle passive range of motion, supporting the cat in a standing position to retrain normal placement of the pelvic limbs and feet, and very gentle antegrade massage from the feet to the hip. Owners can be trained how to administer rehabilitation therapy, which can be performed every six hours for the first one to two weeks of recovery, then tapered depending on neuromotor status. In between session, cats are at risk of excoriation of the dorsal aspect of the limbs with affected pelvic limbs as these cats tend to move with the distal limbs extended behind them. The legs hence may require dressings to protect them. Limb edema may be observed days after the event as a consequence of severe muscle injury (and predicts a poorer chance for full recovery) and should not be mistaken for an early sign of ATE (Bonagura, 2010). These complications are usually restricted to cats with very severe ischemic damage and it should be advised to recheck these patients every three to four days by the treating veterinarian to reassess limb function and the need for continued analgesia, presence of pulses, control of CHF signs and the absence of possible necrotic changes in the skin.

Conclusively, cats with underlying cardiomyopathy should be re-evaluated on a regular base via ap-

propriated diagnostic modalities, e.g. thoracic radiographs and echocardiographic examination, and a therapy adjusted accordingly.

PREVENTION OF ATE

Given the dramatic presentation and guarded prognosis, prevention of an ATE event is clearly preferable. As already mentioned, cardiomyopathy is the most prevalent etiological cause, and ATE prevention is a standard part of cardiac patients with increased risk. However, cats with non-cardiac diseases predisposing them to ATE, such as hyperthyroidism, protein-losing enteropathy or nephropathy (rare in cats) and pulmonary neoplasia, may also benefit from thromboprophylaxis in specific situations.

Primary prevention

Primary prevention focuses on preventing an initial ATE event in cats that appear to be at risk; however, at this time, no studies on primary prevention of CATE in veterinary medicine are available. In some studies, it has been tried to define risk factors for the development of CATE. In a retrospective study on cats with HCM, it has been demonstrated that cats presenting with ATE have a significantly larger left atrial size, end-systolic left ventricular diameter and lower fractional shortening than asymptomatic cats or cats with CHF (Payne et al., 2010). Based on this report, some specialists recommend primary prevention in cats with an end-systolic diameter greater than 1.7 cm, a left atrium-to-aortic ratio greater than 2.0 or auricular emptying velocities of <20cm/s. Primary prevention is also indicated in cats with spontaneous contrast or 'smoke' in the left atrium on echocardiography (Schober and Maerz, 2006).

Secondary prevention

Secondary prevention focuses on preventing a subsequent ATE event in cats that have a history of ATE. In several studies, a recurrence rate of cats receiving some antithrombotic therapy has been reported from 17% to 75%, with a one-year recurrence rate of 25% to 50%. The FATCAT study has shown clopidogrel to be superior to aspirin with a ATE recurrence rate of 49% (versus 75% for aspirin) and a one-year recurrence rate of 36% (versus 64% for aspirin). Clopidogrel was also associated with longer median time to ATE event (443 days) than aspirin (192 days) (Hogan et al., 2015). Cats that have already experienced an ATE event have an objectively demonstrated increased risk, so prevention is recommended in all cats with an objective or suspected history of ATE.

CONCLUSION

Feline arterial thromboembolism is thought to be an uncommon disease, with a prevalence ranging from 0.3-0.6% in practice (Smith et al., 2003; Borgeat et al., 2014). Despite the low prevalence, it is considered to be one of the most distressing conditions encountered in feline practice, because of the lack of preceding signs, the devastating clinical presentation and the generally poor prognosis. Given these conditions, the majority of these affected cats are currently euthanized at the time of diagnosis. Although the clinical signs of CATE are dramatic and acute survival is relatively low, the majority of these cats are fairly stable within 48-72 hours. Therefore, owners should consider therapy for at least the first 72 hours and not make a decision for immediate euthanasia (Hogan, 2017). Despite the generally poor prognosis, with survival rates ranging from 33% to 39%, a subpopulation of these patients may have long-term survival. Some cats survive over a year with a satisfying quality of life, in case of adequate treatment and follow-up. Affected cats may display positive prognostic factors indicative of higher survival rate and better mid- to long-term prognosis, namely the absence of CHF, rectal temperature at the moment of presentation >37.2°C, (partial) presence of motor function, and the site of thromboembolism and associated affected limbs or other organs (Schoeman, 1999; Borgeat et al., 2014). A correct identification of specific prognostic factors obtained from results of physical examination and further testing, enables the veterinarian to give the owner the best possible advice regarding further treatment, management and prognosis. Current treatment should always consist of initial clinical stabilization with adequate and adjusted analgetic and antithrombotic treatment.

Furthermore, diuretic therapy should be administered if concomitant CHF is diagnosed. At the moment, there is no fixed treatment protocol, several drug classes can be considered and treatment approach should always be evaluated individually. Prospective studies regarding the primary prevention of ATE events are currently lacking in feline medicine, but for secondary prevention, clopidogrel has been shown superior to aspirin (Hogan et al., 2015). Conclusively, further studies are needed to investigate possible alternative therapies and preventive measurements.

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The full reference list may be obtained from the lead author on request.

SYMPTOMATISCHE BEHANDELING VAN *E. COLI*-MASTITIS

VRAAG

"Sinds de strengere wetgeving rondom het gebruik van antibiotica en het selectief droogzetten zien we een stijging van het aantal koeien met ernstige coli-mastitis. Ik zou graag willen vragen hoe en met welke middelen ik zulke koeien er door kan helpen, rekening houdend met het antibiotcabeleid in Nederland. Wat me vooral interesseert zijn de behandelingen zonder antibiotica, zoals pijnstilling, infuus en drenchen."

ANTWOORD

Escherichia coli (*E. coli*) veroorzaakt typisch een acute, klinische uierontsteking waarbij de koe erg ziek kan zijn. De kiem infecteert de uier via het tepelkanaal, voornamelijk bij het begin van de droogstand, vóór de vorming van een keratineplug het slotgat afsluit en tenslotte rond de periode van het afkalven wanneer de keratineplug begint te verweken. Voornamelijk hoogproductieve koeien op het moment van afkalven en in het begin van de lactatie zijn gevoelig voor deze omgevingsgebonden kiemen (Burvenich et al., 2003). Bij het gebruik van een antimicrobieel middel moet men voor een breedspectrumantibioticum kiezen. Ideaal wordt de beslissing gemaakt op basis van een antibiogram van de oorzakelijke kiem. Indien deze gegevens niet voorhanden zijn en rekening houdend met het antibioticabeleid in Nederland wordt de klinische uierontsteking het beste systemisch behandeld met een hoge dosis trimethoprim-sulfonamiden om de twaalf uur bij het vermoeden of ter preventie van bacteriëmie en eventueel intramammair met een breedspectrumantibioticum (tweede keuze) (Suojala et al., 2013).

De klinische symptomen worden echter voornamelijk veroorzaakt door endotoxinen, waardoor ondersteunende therapie doorslaggevender is, zoals een anti-inflammatoire behandeling: NSAID's of corticosteroïden (Burvenich et al., 2003). Bij vroegtijdig gebruik verminderen de klinische symptomen, het productieverlies en verhogen de genezingskansen. De koe dient regelmatig leeggemolken te worden, idealiter om de twee uur en eventueel na toediening van oxytocine (Suojala et al., 2010). Bij shockverschijnselen is infuustherapie van een hypertone zoutoplossing aangewezen samen met orale rehydratatie (20 tot 40 l). Van een 7,2% NaCl-oplossing mag 4 tot 5 ml/kg lichaamsgewicht toegediend worden aan een snelheid van $1ml.kg^{-1}.min^{-1}$ (Constable, 2003). Indien op basis van de symptomen hypocalcemie wordt vermoed, dient een calciuminfuus toegediend te worden onder zorgvuldige cardiale monitoring. Hoe sneller de behandeling ingesteld wordt, hoe groter de kans op genezing (Anderson, 1989).

Verminderd en verantwoord antibioticagebruik is een van de actiepunten in de strijd tegen verworven antimicrobiële resistentie (WHO, 2015). In Nederland is preventief gebruik van antimicrobiële middelen bij het droogzetten van koeien niet meer mogelijk. Het selectief droogzetten van dieren is de richtlijn geworden. Enkel koeien waarbij na diagnostisch onderzoek een intramammaire infectie vermoed wordt, mogen nog behandeld worden met een langwerkend antibio-ticum. De keuze van een antibioticahoudend droogzetpreparaat is tevens gereglementeerd (KNMvD, 2013). Het gebruik van een inwendige tepelafsluiter, een uierpreparaat zonder antimicrobiële bestandsdelen, is wel nog toegelaten. Deze speenafsluiter sluit de uier af van de omgeving met een siliconeprop en verlaagt zo de kans op nieuwe infecties met onder andere *E. coli*. De speenafsluiter dient correct ingebracht te worden: uitmelken, speenafsluiter ontluchten, speentoppen zorgvuldig ontsmetten en bij het inbrengen de speenbasis afnijpen zonder de inhoud in de uier te masseren (Huxley et al., 2002). De artificiële prop is niet bestand tegen een verhoogde melkdruk en zal verwijderd worden bij melklekken (Zobel et al., 2013). Naast een goede droogzettherapie zijn een droge en propere huisvesting voor drachtige vaarzen, droogstaande koeien en een goede hygiëne van de afkalfbox van groot belang om besmettingen met omgevingskiemen, zoals *E. coli* te voorkomen (Barkema et al., 1999).

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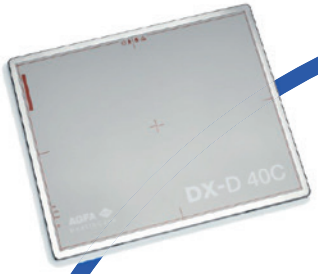

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