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UNIVERSITEIT GENT

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(24 - 36 uur per week)

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WIJ VRAGEN

Voor deze functie van dierenarts zoeken wij een enthousiaste kandidaat die flexibel inzetbaar is. Ervaring als dierenarts gezelschapsdieren is een pré, maar niet noodzakelijk. Wij zoeken een collega die past in het team GD-dierenartsen, die klantgericht werkt en veterinaire kwaliteit nastreeft. Wonen in het werkgebied is belangrijk, of de bereidheid om hier naartoe te verhuizen. Jouw werkzaamheden vinden plaats op zowel onze hoofdlocatie in Vorden, als op een van de dependances. Deelname in de weekenddienstregeling is een vereiste, de invulling ervan leggen we je graag uit in een gesprek.

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VLAAMS DIERGENEESKUNDIG TIJDSCHRIFT

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INHOUD

Coverfoto: Martine Van Den Broeck

Tijdens de lockdown-periode van de coronacrisis is de vraag naar pups enorm toegenomen. Niet alleen was er door het verplichte thuiszitten meer tijd om met de nieuwe pup bezig te zijn, gaan wandelen met de hond vormde een ideaal excuus om toch naar buiten te kunnen. Het is maar te hopen dat al deze pups na de coronacrisis nog steeds even welkom zijn. De vraag naar pups overstijgt echter al jaren het aanbod op de binnenlandse markt. In Vlaanderen is daardoor bijna één op vijf van de geregistreerde pups geïmporteerd vanuit het buitenland. Er wordt vermoed dat deze handel niet altijd correct verloopt en dat deze pups veel jonger zijn dan de wettelijk toegelaten leeftijd. Om dit te kunnen controleren is een betrouwbare leeftijdsschatting nodig. Voor de leeftijdsperiode tussen acht weken (speenleeftijd) en vijftien weken (importleeftijd) komen momenteel gebitscontrole en radiografie van de beenderen het meest in aanmerking.

Tekst: Martine Van Den Broeck

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Nadia Eeckhout
Salisburylaan 133, B-9820 Merelbeke
Tel. 09 264 75 13
nadia.eeckhout@UGent.be

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Information (for authors) and Subscriptions:
Nadia Eeckhout
Salisburylaan 133, B-9820 Merelbeke
Tel. 09 264 75 13
nadia.eeckhout@UGent.be

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De literatuuraangave **in de tekst** dient als volgt te gebeuren: de naam van de auteur(s) en het jaar van publicatie (Voorbeeld: "… werd vroeger aangetoond (Brown, 1975; Brown en Ellis, 1975; Brown *et al.*, 1975)" ofwel "Brown (1975) toonde vroeger aan dan …". Er is dus geen cijferaanduiding in de tekst.
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Figuren en tabellen dienen contrastrijk te zijn en op afzonderlijke bijlagen te worden ingediend. De figuren moeten een grootte hebben van minstens 200 kb.
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Overzichtsartikelen mogen niet te uitgebreid zijn (norm: max. 20 getypte bladzijden) en het aantal referenties wordt beperkt gehouden.

De auteurs gaan ermee akkoord dat hun gepubliceerd artikel hergebruikt kan worden, mits vermelding van de bron.

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Legal framework and current techniques for age estimation in puppy trade

Wettelijk kader en huidige technieken voor leeftijdsschatting in de puppyhandel

M. Van den Broeck, P. Cornillie

Department of Morphology, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

martine.vandenbroeck@ugent.be

ABSTRACT

Dog breeding and dog trade are strictly regulated by EU-legislation, as well as by national and regional legislations. Nevertheless, it is believed that part of the puppies traded within the EU are younger than the legal minimum age, with the risk of compromising their own health and wellbeing as well as public health. A proper age determination is necessary to expose potential malpractices. Combining multiple independent methods is essential to compensate for possible variations in one single estimation method. Assessment of the dental age and bone age, more specifically by evaluating the eruption status of the dentition and the radiographic appearance and formation of ossification centers are both eligible. However, current available data are not detailed enough. In order to improve the accuracy and reduce the error in age estimation, more breed specific data are required, more intermediate development stages must be described and possible developmental disorders and pathologies must be considered.

SAMENVATTING

Hondenfokkerij en -handel zijn strikt gereguleerd door EU-wetgeving en nationale en regionale wetgeving. Desondanks wordt aangenomen dat een deel van de verhandelde puppy's binnen de EU jonger is dan de wettelijke minimumleeftijd. Dit kan zowel de gezondheid en het welzijn van de pup als de volksgezondheid in gevaar brengen. Een juiste leeftijdsbepaling is noodzakelijk om mogelijke inbreuken aan te tonen. Het combineren van meerdere onafhankelijke methoden is essentieel ter compensatie van mogelijke variaties binnen één methode. Het vaststellen van zowel de gebits- als de botleeftijd, meer specifiek aan de hand van de eruptiestatus van het gebit en de radiologische ontwikkeling van ossificatiecentra, komen hiervoor in aanmerking. De huidige beschikbare gegevens zijn echter niet gedetailleerd genoeg. Om de nauwkeurigheid van de leeftijdsschatting te verhogen en fouten te vermijden zijn 1. meer rasspecifieke gegevens noodzakelijk, 2. moeten meer tussenliggende ontwikkelingsstadia beschreven worden en 3. moet rekening gehouden worden met mogelijke ontwikkelingsstoornissen en afwijkingen.

INTRODUCTION

According to the Fediaf report of 2019, an estimated 25% of the European Union households owns at least one dog. The total number of owned dogs reaches 65.5 million (Fediaf, 2019). The figures for Belgium are comparable and have remained stable since 2012 at about 24% of the households (Van Bogaert, 2013; Fediaf 2017 – 2019; Statista 2019), with an estimated total of 1.3 million dogs (Fediaf, 2019). Of the 97.437 newly registered dogs in Flanders in 2018, 18.546 came from abroad (19%) (Weyts, Flemish Parliament, 2019). A considerable part of these pups was imported from Eastern European countries. The main countries were: Slovakia (11.570; 59%), the Czech Republic, (3.932; 20%), Hungary (1.812;

Table 1. Overview of EU-, Belgian and Flemish legislation relating to animal welfare, dog breeding and sale.

Domain	Law/Regulation	Published	Full Title + Internet link
General			
EU	European Convention for the Protection of Pet Animals	13/11/1987	European Convention for the Protection of Pet Animals https://www.coe.int/en/web/conventions/full-list/-/conventions/treaty/125
	Protocol on Animal Welfare in the Treaty of Amsterdam	10/11/1997	Protocol (No 33) on protection and welfare of animals (1997) annexed to the Treaty establishing the European Community (consolidated version) http://data.europa.eu/eli/treaty/tec_2006/pro_33/oj
	Article 13 of the Treaty of Lisbon	17/12/2007	Article 13 of the Treaty of Lisbon amending the Treaty on the European Union and the Treaty establishing the European Community, signed at Lisbon, 13 December 2007 http://data.europa.eu/eli/treaty/lis/sign
	Council Directive 92/65/EEC	14/09/1992	Council Directive 92/65/EEC of 13 July 1992 laying down animal health requirements governing trade in and imports into the Community of animals, semen, ova and embryos not subject to animal health requirements laid down in specific Community rules referred to in Annex A (I) to Directive 90/425/EEC http://data.europa.eu/eli/dir/1992/65/2014-12-29
Belgium	Animal Welfare Law 1986		Law on the Protection and Welfare of Animals of August 14, 1986 http://www.ejustice.just.fgov.be/eli/wet/1986/08/14/1986016195/justel
Trade			
EU	Council Regulation (EC) 1/2005	05/01/2005	Council Regulation (EC) No 1/2005 of 22 December 2004 on the protection of animals during transport and related operations and amending Directives 64/432/EEC and 93/119/EC and Regulation (EC) No 1255/97 http://data.europa.eu/eli/reg/2005/1/oj
	Regulation (EU) 576/2013	28/06/2013	Regulation (EU) No 576/2013 of the European Parliament and of the Council of 12 June 2013 on the non-commercial movement of pet animals and repealing Regulation (EC) No 998/2003 http://data.europa.eu/eli/reg/2013/576/oj
	Commission Implementing Regulation (EU) 577/2013	28/06/2013	Commission Implementing Regulation (EU) No 577/2013 of 28 June 2013 on the model identification documents for the non-commercial movement of dogs, cats and ferrets, the establishment of lists of territories and third countries and the format, layout and language requirements of the declarations attesting compliance with certain conditions provided for in Regulation (EU) No 576/2013 of the European Parliament and of the Council http://data.europa.eu/eli/reg_impl/2013/577/2014-12-29
	Commission Implementing Decision (EU) 2013/518	23/10/2013	Commission Implementing Decision of 21 October 2013 amending Part 1 of Annex E to Council Directive 92/65/EEC as regards the model health certificate for animals from holdings http://data.europa.eu/eli/dec_impl/2013/518/oj

	Commission Delegated Regulation (EU) 2018/772	28/05/2018	Commission Delegated Regulation (EU) 2018/772 of 21 November 2017 supplementing Regulation (EU) No 576/2013 of the European Parliament and of the Council with regard to preventive health measures for the control of Echinococcus multilocularis infection in dogs, and repealing Delegated Regulation (EU) No 1152/2011, C/2017/7619 http://data.europa.eu/eli/reg_del/2018/772/oj
	Commission Implementing Regulation (EU) 2018/878	19/06/2018	Commission Implementing Regulation (EU) 2018/878 of 18 June 2018 adopting the list of Member States, or parts of the territory of Member States, that comply with the rules for categorisation laid down in Article 2(2) and (3) of Delegated Regulation (EU) 2018/772 concerning the application of preventive health measures for the control of Echinococcus multilocularis infection in dogs http://data.europa.eu/eli/reg_impl/2018/878/oj
	Commission Implementing Decision (EU) 2019/294	20/02/2019	Commission Implementing Decision (EU) 2019/294 of 18 February 2019 laying down the list of territories and third countries authorised for imports into the Union of dogs, cats and ferrets and the model animal health certificate for such imports (notified under document C(2019) 1059). C/2019/1059. http://data.europa.eu/eli/dec_impl/2019/294/oj
	Commission Implementing Decision (EU) 2019/1206	16/07/2019	Commission Implementing Decision (EU) 2019/1206 of 12 July 2019 amending Part 1 of Annex E to Council Directive 92/65/EEC as regards the animal health certificate for trade in dogs, cats and ferrets (notified under document C(2019) 5210) http://data.europa.eu/eli/dec_impl/2019/1206/oj
Belgium	RD 29/10/2014	29/12/2014	Royal Decree on the animal health requirements for the movement of dogs, cats and ferrets http://www.ejustice.just.fgov.be/eli/besluit/2014/12/13/2014024410/staatsblad
	RD 18/09/2016	17/10/2016	Royal Decree on the prevention and control of rabies http://www.ejustice.just.fgov.be/eli/besluit/2016/09/18/2016024202/staatsblad
Breeding and Trade			
Belgium	RD 28/05/2004 Modified by RD 25/04/2014	07/06/2004 27/06/2014	Royal Decree on the identification and registration of dogs http://www.ejustice.just.fgov.be/eli/besluit/2014/04/25/2014024260/staatsblad
	RD 27/04/2007	06/07/2007	Royal Decree laying down the conditions for approval of establishments for animals and the conditions for the marketing of animals http://www.ejustice.just.fgov.be/eli/besluit/2007/04/27/2007022825/staatsblad
Flanders	FC 15/02/2019	11/04/2019	Decree of the Flemish Council to amend various provisions of the Royal Decree of 27 April 2007 concerning the conditions for approval of establishments for animals and the conditions regarding the marketing of animals http://www.ejustice.just.fgov.be/cgi/article_body.pl?language=nl&caller=summary&pub_date=19-04-11&numac=2019011560

9.24%), Spain (1.104; 5.6%) and Romania (1.016; 5.18%) (Ponsaerts, 2019). The annual value of cat and dog sales in the EU is estimated at 1.3 billion euro, generating direct employment for 300.000 people (Schrijver et al., 2015).

Buying a puppy is often an impulse purchase (Yeates and Bowles, 2017; Maher and Wyatt, 2019). Consumers are strongly attracted by the 'cute factor' of a puppy, making dogs most marketable at a young age (Yeates and Bowles, 2017; Chersini et al., 2018). According to the British Kennel Club (2017), 36% of the British puppy buyers spend less than twenty minutes researching where to buy their puppy, with the risk of buying from an irresponsible source. Consumers who do not want to be put on a waiting list or who do not want to adopt a shelter dog, often turn to commercial breeders who have (often cheaper) puppies immediately available. The internet has become the principal source where to look for puppies for sale (Maher and Wyatt, 2019). It is more accessible, makes a rapid purchase possible and for many people comes across as more reliable (Wyatt et al., 2017). Puppy trade in Belgium is strictly regulated. In Flanders, only officially recognized breeders with a valid approval number can advertise on trading websites or social media. (Private) persons without approval number can only advertise via specialized media (specialized magazines or websites) (Animal Welfare Law, 2016), and are prohibited to sell dogs or give away for free via social media (official website Flemish Government, 2020). Compliance to these regulations is however very difficult to control (Lavorgna, 2015; Yeates and Bowles, 2017). Puppies are offered for sale in private groups or personal messages on facebook (Hus, 2018). Advertisements on trading websites can be misleading, for example by pretending to sell pups raised in a domestic setting when actually, the pups come from large breeding facilities (Hus, 2018).

Breeding and trading dogs is thus an important economic activity with potentially high profit mar-

Table 2. Different types and number of dog breeders in Flanders (on 28/02/2020) and the most important conditions* they must meet.

Breeder type	Conditions (RD 27/04/07, *adjusted** by FC 15/02/19*)
Occasional breeder (occasionele kweker/ éleveur occasionel) n = ?	• License number (HK number) not obligatory *** • Max. two litters/year • May only advertise in specialized press (not on internet or social media)
Amateur breeder (hobbykweker/ éleveur amateur) n = 732	• License number (HK number) obligatory • Must issue a two-year guarantee certificate • *Max. five litters/year (1)* • *Max. three breeds (2)* • Veterinary check at least two times/litter • Can advertise on the internet if license number is stated. *Ads with photos of animals other than the animals offered are not permitted*
Professional breeder (beroepskweker/ éleveur professionnel) n = 122	• License number (HK number) obligatory • Trades only dogs from own breeding • Must issue a two-year guarantee certificate • *Can have more than five litters/year (1)* • *Max. seven breeds (from 01/01/2021) (2)* • *The responsible person or a permanent staff member has a recognized diploma or certificate (from 01/01/2024)* • *Veterinary check at least once a month and at least two times/litter* • Can advertise on the internet if license number is stated. *Ads with photos of animals other than the animals offered are not permitted*
Breeder-merchant (kweker-handelaar/éleveur commerçant) n = 46	• License number (HK number) obligatory • Trades dogs from own breeding and from other facilities • Must issue a two-year guarantee certificate • At least ten litters of own breeding/year • Max. seven own breeds (from 01/01/2021) (2) • Puppies from other facilities are quarantined for at least ten days • Veterinary check at least once a month and at least two times/litter • The responsible person or a permanent staff member has a recognized diploma or certificate (from 01/01/2024) • Can advertise on the internet if license number is stated. *Ads with photos of animals other than the animals offered are not permitted*

* In addition to animal welfare regulations, dog breeders must also comply with environmental legislation: for keeping more than five 'dogs', a class 3 environmental license application is required (notification obligation), for keeping more than ten 'dogs', a class 2 environmental license application is required (license obligation) (Flemish environmental legislation VLAREM II). In this regulation, the term 'dogs' only applies to dogs older than six months.
** These adjustments do not apply for breeders in Wallonia and Brussels, where - at the moment- the original conditions laid down in the royal Decree of 27 April 2007 still apply: (1) amateur breeder max. ten litters/year and professional breeder more than ten litters/year; (2) no restrictions on the number of dog breeds.
*** In Wallonia an occasional breeder - approval is obligatory (Decree of the Walloon Government of 11 May 2017).

gins, which, combined with consumer buying behavior, can easily result in animal welfare being of secondary importance.

LEGAL ASPECTS CONCERNING DOG BREEDING AND TRADE

A list of EU-, Belgian and Flemish legislations relating to animal welfare, dog breeding and sale can be found in Table 1.

Animals are recognized as sentient beings in European Union legislation (Protocol on Animal Welfare in the Treaty of Amsterdam, 1997; Article 13 in the Treaty on the Functioning of the EU or the Treaty of Lisbon, 2007). Very recently, also the Belgian Federal Parliament has adopted a revised Civil Code, recognizing animals as sentient beings (Chamber of the Belgian Federal Parliament, 2020; Weyts, Flemish Parliament, 2020). Although symbolically important, this recognition does not form any legal basis for animal welfare legislation. In legal terms, animals remain under the category of 'legal objects' and are considered as property subjected to property laws (Simonin and Gavinelli, 2019), albeit with special consideration on account of their well-being (Francione and Kunstler, 1994). At European level, general regulations for the protection of animal welfare were laid down in the European Convention for the Protection of Pet Animals of 13 November 1987 (Treaty 125 of the Council of Europe), ratified by Belgium in 1991. Specific measures are only formulated in EU legislation to ensure animal welfare during commercial transport (Council Regulation (EC) 1/2005). Although binding and applicable in all EU member states, there is little evidence that this regulation is equally being enforced (FECAVA et al., 2016). The responsibility for keeping and breeding pet animals remains with the national legislation of the individual member states (Schrijver et al., 2015) and may differ enormously amongst the member states (EU dog and cat alliance, 2015).

In Belgium, the Law on the Protection and Welfare of Animals of August 14, 1986 (the Animal Welfare Law) forms the basis for all other regulations that govern welfare in dog breeding and dog trade. Because of the regionalization of animal welfare in Belgium, different regulations may apply for the Flemish, Walloon and Brussels capital region.

Since October 1, 2019, the legal minimum age at which puppies can be weaned in Flanders, has increased from seven to eight weeks (FC 15/02/2019), out of consideration that the nesting period is a very important phase for the development and socialization of puppies. Puppies should stay long enough in a familiar environment with their mother and littermates. 'Long enough' may differ among breeds (Scott and Fuller, 1965; Morrow et al, 2015) but the maternal period should at least last until natural weaning occurs (Wilsson, 1984; Case, 2005). Early weaning interferes with the behavioral development (Elliot and Scott, 1961; Overall, 2013; McMillan et al., 2013; McMillan, 2017) and increases aggressive and abnormal behavior (Pfaffenberger, 1963; Fox and Stelzner, 1966; Lindsay, 2000; Pierantoni et al., 2011; Tiira et al., 2012). All puppies must also have had at least a primo vaccination against parvovirus, distemper virus, Bordetella and para-influenza virus (kennel cough) and hepatitis contagiosa canis virus before being sold (FC 15/02/2019) and must be correctly identified. Dogs in Belgium have to be identified with a microchip and have to be registered in the central database DogID (https://dogid.be). The identification has to be linked to a European pet passport (for dogs born after June 7th, 2004). Puppies born in Belgium have to be identified before the age of eight weeks (RD 28/05/2004 and RD 25/04/2014).

Breeding facilities can range from occasional breeders selling only once or twice a litter, to commercial breeders selling thousands of pups a year. In Table 2, the different types of dog breeders in Belgium, in accordance to the royal decree (RD 27/04/2007) with the latest adjustments for Flanders (FC 15/02/2019) are shown. There are four types of breeders: the occasional breeder, the hobby breeder, the professional breeder and the breeder-merchant. Although dogs are now considered as sentient beings in the Belgian Civil Code, the sale of dogs still falls under the consumer protection law (consumer protection law of September 1, 2004). This means, among other things, that a two-year guarantee certificate must be issued by all legally recognized establishments (i. e. all but occasional breeders). Breeder merchants mostly resell pups from other breeders but are required to breed a minimum of ten own litters every year. The puppies they buy for further sale can either come from local breeders - occasional, hobby or professional - or from foreign breeders, provided that these breeders meet the required standards (recognized foreign breeders and breeders from countries whose legislation lays down at least the same conditions) (ad III(2) of RD 27/04/2007). The Belgian governments use a list of registered breeding establishments in Eastern European countries that meet these standards, although part of the breeders on the list are collection centers that resell local pups to Western European countries (Weyts, Flemish Parliament, 2015; Weyts, Flemish Parliament, 2018). Compliance to the established conditions is checked by veterinarians of the exporting countries (Flemish Department of Animal Welfare, 2020).

The import and export of dogs to and within the EU are subjected to specific regulations that are implemented in Belgium by the Royal Decree of 13/12/2014. Distinction is made between commercial and non-commercial movements and between intra-union trade and import from non-European countries. Transports are considered commercial when 1. the owner or a legal representative of the owner is travelling more than five days before or after the movement of the pet, 2. when the purpose of the transport invol-

ves a sale or transfer of ownership or 3. when more than five animals are traveling with or without their owner (Regulation (EU) 576/2013).

As already mentioned, the welfare of animals during transport is regulated by Council Regulation (EC) 1/2005. All dogs crossing borders have to be identified by microchip, accompanied by a valid (European) Passport (Regulation (EU) 576/2013 and Commission Implementing Regulation (EU) 577/2013) and have to be at least fifteen weeks old. The latter results from the requirement that all dogs crossing borders should be correctly vaccinated against rabies (Regulation (EU) 576/2013, Directive 92/65/EEC and Commission Implementing Decision (EU) 2013/518). This prerequisite is in correspondence with the protocol of the anti-rabies vaccine manufacturers who state that a primo-vaccination only establishes a protective immunity when it has been administered at the age of at least twelve weeks, after which a three-week waiting period is taken into

Table 3. List of the prerequisites and legislation for the commercial and non-commercial transport of dogs.

Types of transport	EU	non-EU
Non-commercial	Regulation (EU) 576/2013 and Commission Implementing Regulation (EU) 577/2013 • Must be identified with a microchip • Must be accompanied by a valid pet passport Regulation (EU) 576/2013 and Council Directive 92/65/EEC implemented by Commission Implementing Decision (EU) 2013/518 • Must be correctly vaccinated against rabies - primo vaccination earliest at twelve weeks - a three-weeks waiting period	Regulation (EU) 576/2013 and Commission Implementing Regulation (EU) 577/2013 • Must be identified with a microchip • Must be accompanied by a valid pet passport Regulation (EU) 576/2013 and Council Directive 92/65/EEC implemented by Commission Implementing Decision (EU) 2013/518 • Must be correctly vaccinated against rabies - primo vaccination earliest at twelve weeks Additionally: Regulation (EU) No 576/2013 • Rabies antibody titration test at least thirty days after vaccination and at least three months before transport Regulation (EU) No 576/2013, Regulation (EU) No 577/2013) • Must be accompanied by a health certificate by an authorized veterinarian and a written declaration concerning non-commercial nature • Must cross borders through a travellers' point of entry
Commercial	Regulation (EU) 576/2013 and Commission Implementing Regulation (EU) 577/2013 • Must be identified with a microchip • Must be accompanied by a valid pet passport Regulation (EU) 576/2013 and Council Directive 92/65/EEC implemented by Commission Implementing Decision (EU) 2013/518 • Must be correctly vaccinated against rabies - primo vaccination earliest at twelve weeks - a three-weeks waiting period Additionally: Council Directive 92/65/EEC • Must come from registered holdings Council Regulation (EC) No 1/2005, Council Directive 92/65/EEC implemented by Commission Implementing Decision (EU) 2013/518 • Must be registered in TRACES (Trade Control and Expert System) • Must be accompanied by a health certificate completed and issued by an authorized veterinarian within 48 hours prior to transport	Regulation (EU) 576/2013 and Commission Implementing Regulation (EU) 577/2013 • Must be identified with a microchip • Must be accompanied by a valid pet passport Regulation (EU) 576/2013 and Council Directive 92/65/EEC implemented by Commission Implementing Decision (EU) 2013/518 • Must be correctly vaccinated against rabies - primo vaccination earliest at twelve weeks Additionally: Regulation (EU) No 576/2013 • Rabies antibody titration test at least thirty days after vaccination and at least three months before transport Regulation (EU) 576/2013; Commission Implementing Regulation (EU) 577/2013 and Commission Implementing Decision (EU) 2019/294 • Must come from listed authorized territories or third countries • Must be accompanied by a health certificate completed and issued by an authorized veterinarian within 48 hours prior to transport • Must be subjected to specific controls at veterinary border inspection posts

For Malta, United Kingdom, Ireland and Finland, additional prerequisites must be met concerning treatment against *Echinococcus multilocularis* (Commission Delegated Regulation (EU) 2018/772 and Commission Implementing Regulation (EU) 2018/878). Currently, in the transition period after Brexit, all previous legislation for pets traveling to the UK and between the UK and the EU remains in effect.

account. Since Belgium has officially been declared free from rabies since 2012 (FAVV, 2018), dogs, cats and ferrets only have to be vaccinated against rabies if they cross borders. Problems may arise in the non-vaccinated population within the country if non-vaccinated or badly vaccinated animals are imported from an endemic area, posing a serious risk to public health. Specific regulations regarding the prevention and control against rabies in Belgium are regulated in the Royal Decree on the prevention and control of rabies (RD 18/09/2016). More regulations apply for commercial transports or when the transport concerns the import from a non-EU-country. A summary of these prerequisites and legal texts involved can be found in Table 3.

ILLEGAL DOG TRADE

It is believed that a considerable part of the puppies traded to and in between countries of the European Union comes from irresponsible or malicious breeders (FECAVA et al., 2016). It is said that many of these pups and their mothers are kept in sickening conditions (McMillan et al., 2011; Yeates and Bowles, 2017). The illegal puppy trade has many faces and does not only concern cross border smuggling of hidden, non-registered pups or undeclared commercial transports slipping past inspection posts; it also involves the breeding and selling of more litters than licensed to, the excessive breeding of dams, the selling of underaged puppies (whether or not with falsified passports), and the forging or manipulation of official documents of pups in declared transports to meet the legally required conditions (Van Uhm, 2010; Four Paws International, 2013; Posch et al., 2013; Hus, 2017; Kuijpers, 2017; Wyatt et al., 2017, DogsTrust, 2018). In case underaged puppies are imported, they may have been weaned too early and may not have been vaccinated correctly, compromising their behavioral development and own health and potentially causing serious threats to human health.

IMPORTANCE OF AGE ESTIMATION

Being able to estimate the age of pups as accurately as possible is becoming increasingly important and can be a necessary tool to demonstrate potential malpractice in the prospering trade of puppies, with special focus on the age around eight weeks and around fifteen weeks. When there are doubts on the certainty of the 'alleged' age, an independently performed age assessment preferably based on a combination of several reliable methods, can give a reliable estimation. This can help official bodies to check compliance with legal provisions. It can help veterinarians in performing a better health control and, for example, apply a vaccination protocol adapted to the actual age. Puppy traders who buy foreign puppies for further sale are offered the opportunity to refuse puppies when it can be demonstrated without any doubt that these puppies are too young.

TECHNIQUES FOR AGE ESTIMATION IN PUPS

General morphological and behavioral development

Puppies go through specific developmental stages recognizable by a characteristic appearance and behavior. The major part of these developmental characteristics can only give a preliminary, rough estimation of age (Scott and Fuller, 1965; Markwell and Thorne, 1987; Serpell et al., 1995; Overall, 2013). Many obvious sensory and motor developmental milestones, such as the opening of the eyelids and ear canals or learning to walk and bark, already occur in the first weeks of life (Scott, 1958; Fox, 1964; Mosier, 1978; Hoskins and Parkington, 2001; Landsberg, 2001; Lavely, 2006), making them unusable for age assessment of pups in transport. Other features, such as growth and the shedding of the puppy coat, continue over a longer period, making the pinpointing of an exact age less probable. Moreover, the age of onset and duration can be breed-, sex- and environmental dependent (Markwell and Thorne 1987; Appleby et al., 2002; Peterson, 2011; Evans and de Lahunta, 2013a; Morrow et al., 2015). The same applies to behavioral development or the first display of a specific behavior (Svartberg, 2006; Kerswell et al., 2010; Morrow et al., 2015; Lenkei et al., 2019).

Dental age

Historically, the first method of choice for age estimation in dogs is based on dental morphology and development (Girard, 1834; Cornevin and Lesbre, 1894; Cadiot and Breton, 1924; Barton, 1939; Piérard, 1967; Habermehl, 1975; Gesierich, 2015). The alleged age is further referred to as dental age, by analogy with human sciences (forensics, archeology and dentistry) (Sehrawata and Singh, 2017; Uzuner et al., 2017; Adserias-Garriga, 2019).

Most commonly, dental age assessment is performed by visual evaluation of tooth eruption (Figure 1). This technique is easily applicable and can be immediately carried out without the need for specific technical equipment or having to sedate the puppy (Piérard, 1967; Gesierich, 2015; Modina et al., 2019). Dental eruption occurs in the time frame of interest. However, intervals in veterinary (anatomy or dentistry) handbooks (Pavaux, 1944; Nickel et al., 1975; Dyce et al., 1991; Lobprise, 1993; Barone, 1997; Hale, 2005; Evans and de Lahunta 2013b; Shoe et al., 2018) are often generalized or even simplified for easy remembering. For forensic purposes, more specific or detailed references, such as Mellanby, 1929; Arnall,

Figure 1. Permanent tooth eruption in an eighteen-week-old Irish setter puppy. The permanent first incisors are almost in occlusion and a tip of the central cusp of the second incisors is visible. Note that the deciduous second incisor of the right mandible is still present.

1961; Kremenak, 1967; Kremenak, 1969; Shabestari et al., 1967) should be used (Table 4). The normal eruption time can be disturbed as a result of primary defects in the eruption process, mechanical obstruction or displacement of a tooth germ (Stapleton and Clarke, 1999). Especially in small and brachycephalic breeds, disorders as delayed or non-eruption can occur and should be kept in mind (Animoto et al., 1992; MacGee et al., 2012).

After the appearance of the complete adult dentition, the degree of tooth wear, in particular of the incisors, can be evaluated. (Girard, 1834; Cornevin and Lesbre, 1894, Seiferle and Meyer, 1942; Pavaux, 1944; Piérard, 1967: Habermehl, 1975; Barone, 1997). However, tooth wear is highly individually variable and depends on factors, such as type of diet and chewing on hard objects (Cornevin and Lesbre, 1894; Seiferle and Meyer, 1942; Piérard, 1967; Habermehl, 1975; Gesierich, 2015) and should consequently not be used anymore.

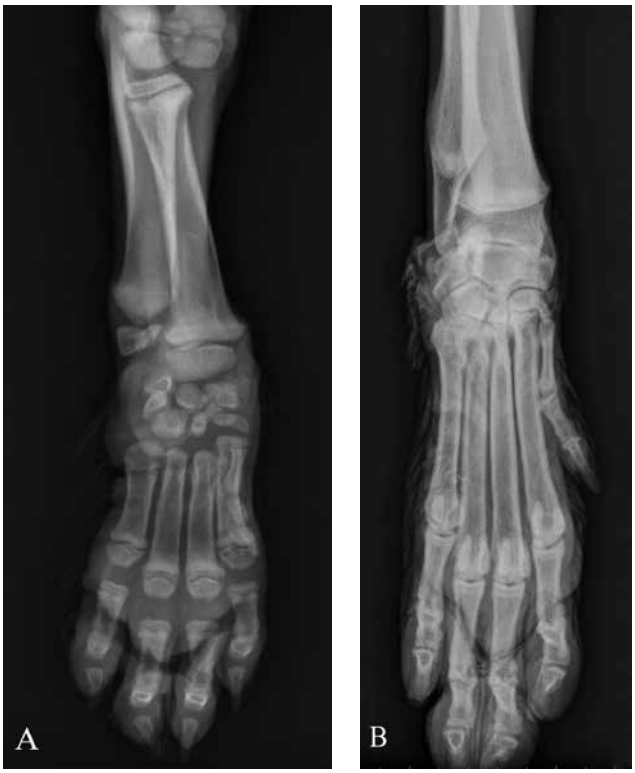
Dental changes can also be registered radiographically. The resorption of deciduous tooth roots (Hooft et al., 1979), the formation of the crown and root of permanent teeth (Hooft et al., 1979, Morgan and Miyabayashi, 1991), the closure of the apical root foramina (Morgan and Miyabayashi, 1991) and the measurement of the pulp cavity diameter (Morgan and Miyabayashi, 1991; De Smet, 2010; Marron et al., 2017) have been described in dogs. Radiographic techniques are however more invasive and (often) require sedation of the pup, technical equipment and more skilled personnel (Holmstrom et al., 2004; Dupont and DeBowes, 2009). Measuring apical root closure and pulp cavity diameter cover an older age range than the one required for pups involved in trafficking (Morgan and Miyabayashi, 1991; De Smet, 2010).

Table 4. Eruption times in days of deciduous and permanent teeth, according to Shabestari et al (1967), Kremenak (1967) and Kremenak (1969). L= Left, R = Right; Mand. = mandible; Max = Maxilla.

	Shabestari, 1967 Beagle n = 106		**Kremenak, 1967 Beagle n = 16 (deciduous); n = 27 (permanent)**		**Kremenak, 1969 Different breeds n= 80**	
	Mand.	**Max.**	**Mand.**	**Max.**	**Mand.**	**Max**
Deciduous						
Id1	29+-3	24+-3	31.5	25.1	L: 30.5+-0.4/R: 30.6+-0.5	L: 23.7+-0.3/R: 23.6+-0.3
Id2	27+-3	23+-3	28	24.2	L, R: 26.0+-0.4	L, R: 22.9+-0.3
Id3	25+-3	23+-3	26.8	24.7	L: 25.3+-0.4/R: 25.2+-0.4	L: 22.5+-0.3/R: 22.5+-0.3
Cd	22+-3	22+-2	22.1	22.6	L, R : 22.8+-0.3	L: 22.6+-0.3/R: 22.7+-0.3
Pd2	31+-4	35+-4	33.6	35.6	L: 33.4+-0.5/R: 32.9+-0.4	L, R: 34.3+-0.4
Pd3	26+-4	29+-4	25.3	29.6	L: 22.6+-0.3/R: 23.1+-0.4	L, R: 28.3+-0.4
Pd4	28+-4	34+-4	28.8	34.7	L: 25.7+-0.3/R: 26.2+-0.3	L, R: 31.9+-0.4
Permanent						
I1	117+-5	115+-6	118.4	117.7		
I2	123+-5	123+-6	125.4	128.3		
I3	134+-6	133+-7	136	137.3		
C	145+-5	146+-7	151.4	154.5		
P1	129+-19	106+-8	119.8	105.2		
P2	149+-10	149+-8	156.8	154		
P3	156+-6	153+-6	158.9	157.5		
P4	155+-7	136+-6	158	139.8		
M1	128+-6	132+-7	132.8	136.2		
M2	149+-6	155+-6	150.4	159.8		
M3	175+-11	/	178.6	/		

Bone age

In human forensics, bone age assessment by radiologically evaluating the skeletal maturity is an important tool for age estimation of children and growing adolescents (Cameron and Martin, 2012; Satoh, 2015). In minors, x-rays of the (left) hand and wrist, the Greulich and Pile-method (Greulich and Pile, 1959) or Tanner and Whitehouse method (Tanner et al., 1975) are most commonly used (Schmeling et al., 2006; Štern et al., 2017), followed by (radiologically or with MRI) assessing the clavicular bone maturation in young adults (Kreitner et al., 1998; Schmeling et al., 2006; De Tobel et al., 2019). In dogs, and by extension in all (domesticated) animals, bone age is hardly used for age determination. Of course, the presence or appearance of ossification centers and the progressive narrowing and subsequent closure of growth plates (on x-rays) (in dogs) has been well-studied, but mainly out of anatomical (Pomriaskinsky-Kobozieff and Kobozieff, 1954; Bressou et al., 1957; Hare, 1959; Hare, 1960; Hare, 1961; Smith and Allcock, 1960; Sumner-Smith, 1966) or clinical interest (Riser, 1973; Gustaffson et al., 1975; Olsson, 1983; Fagin et al., 1992; Breit et al., 2004; Frazho et al., 2010*)*. In veterinary archeologic (Silver, 1969) and forensics publications (Yonamine et al., 1980; Sutton et al., 2018; Modina et al., 2019), the importance of the radiological establishment of the appearance of ossification centers and closure rates of growth plates for age assessment in growing dogs have been pointed out (Figures 2A and B). Reference values in anatomical handbooks (Getty, 1975; De Lahunta and Habel, 1986; Barone, 1996; Dyce et al, 2010) and radiographic handbooks (Carlson, 1967; Ticer, 1975; Schebits and Wilkens, 1986; Kealy et al., 2011; Thrall and Robertson, 2016) are (however) based on various dog breeds making these time frames (possibly) too broad (Sutton et al., 2018, Modina et al., 2019). It should also be kept in mind that factors, such as breed, genetics, nutrition, trauma and disease, can affect skeletal development (Thrall and Robertson, 2016; Sutton et al., 2018).



Figures 2A and 2B. A. Craniocaudal radiograph of the carpus of a ten-week-old Bernese mountain dog puppy and B. A thirty-eight-week-old Bernese mountain dog juvenile. In the puppy, the growth plates are still open and ossification centers, including these of the carpal bones and the distant radial and ulnar epiphyses, are not yet fully developed.

Biological age

Determining the biological age is based on age-related changes in DNA and originates from human aging research, whereby the influence of environmental factors, living conditions, etc. on life expectancy is investigated by comparing biological age with chronical (calendar) age (Jylhävä et al., 2017). In the past decade, multiple methods have been described, extending from determining the telomere length (Mather et al., 2011; Heidinger et al., 2012) to methods as T cell DNA-rearrangement (Zubakov et al., 2010; Ibrahim et al., 2016) and measuring changes in DNA methylation ('epigenetic clock') (Iacobazzia et al., 2013; Jones et al., 2015; Bacalinia et al., 2017). Many studies followed on the application of these molecular techniques in the field of human forensics, ranging from establishing age profiles based on human remains on crime scenes to age assessment in immigration fraud cases (Zubakov et al., 2016; Cassina and Clementi, 2017). Also in canids, DNA methylation has been found to correlate with age (Thompson et al., 2017). Moreover, dogs form a valuable model for human aging due to the similarities in occurring (chronic) diseases and environmental conditions (Hoffman et al., 2018), although they age at a different rate than humans (Wang et al., 2019). Nevertheless, research into the biological age of dogs is still in its infancy, and is, although promising for age estimation within all age categories, not applicable as yet.

DISCUSSION

Age assessment in legal cases requires optimal accuracy, which can be met by combining multiple independent methods in order to compensate for possible variations in any single estimation method (Schmeling et al., 2008). The obtained age interval must not only include the actual age but must also be narrow enough (Adserias-Garriga and Wilson-Taylor, 2019). Possible pitfalls should be well-documented. Not only normally occurring variability should be

considered but also possible developmental disorders or pathologies.

A first impression of the age of puppies can be obtained by the general appearance and behavior of the pup. This is however just an impression and should always be followed by an estimation based on the dental status, which can be immediately and quite easily assessed. Subsequent determination of the radiographic bone age can confirm and even improve the age interval obtained from dental inspection (Van den Broeck and Cornillie, 2018).

The timing of tooth eruption in dogs is breed-dependent, and especially in small and brachycephalic breeds, eruption disorders can occur (Seiferle and Meyer, 1942; Lawson et al., 1967; Habermehl, 1975; Colyer, 1990; Habermehl, 1994; Evans and de Lahunta, 2013b). This should be considered in order to make a reliable dental age estimate. Mostly however, published eruption data do not provide information about the examined breeds or about the number of dogs involved and make no distinction between the first breakthrough or the complete presence of a dental element. In fact, there are few original articles with data obtained from self-conducted research. Other publications contain borrowed data, often with little acknowledgment of the original source. To the authors' knowledge, only Shabestari et al. (1967) and Kremenak (1967, 1969) published extensive data, which are still accessible today. These data are based on studies performed in either beagles or a mixture of breeds. Data published in Colyer's variation of teeth (Colyer, 1990) are derived from Shabestari et al. (1967) and Kremenak (1969), combined with limited studies by Mellanby (1929*)* and Arnall (1961).

Most literature sources concerning the radiographic appearance of ossification centers are based on studies performed in medium to large -sized dog breeds (Modina et al, 2019). In a few recent studies on small breeds, it has been demonstrated that the time period of appearance of ossification centers in these breeds largely corresponds with the time period in medium and large breeds (Di Giancamillo et al., 2016; Modina et al., 2017; Modina et al., 2019). Closure of growth plates is more dependent on the size of the dog and occurs earlier in small breeds, whereas giant breeds can experience delayed closure (Teunissen et al., 2017; Sutton et al., 2018). Since most growth plates are still open around the age of eight to fifteen weeks, bone age estimation in these age periods are mainly based on the appearance and presence of ossification centers, a parameter which is largely breed-independent.

It is a major limitation that most of the current reference data sets for dental age as well as for bone age only describe the timing of the extremes, namely the first emergence and complete presence of a dental element or the first appearance of an ossification center and the time of closure of a growth plate, respectively. The inclusion of intermediate development stages could lead to a more exact age estimate or could narrow down the estimated age range.

CONCLUSION

It is possible to check whether puppies leaving the litter or puppies crossing EU borders have the required minimum age. Dental age assessment in combination with the general physical appearance of the puppies can give a first indication. Subsequent determination of the bone age can provide a more conclusive estimate. If necessary, a second dental and bone age assessment can be performed a week or several weeks later. Current reference values do not suffice and need to be adjusted urgently. It is especially important that more breed-specific data are provided, that more intermediate development stages are described, and that instructions are made available on how to correct for possible deviations.

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An extensive reference list can be obtained upon request.

Transfusies van bloedproducten bij de hond: een retrospectieve studie

Transfusion of blood products in dogs: a retrospective study

M. Boonstra, F. Mortier, S. Marynissen, D. Paepe

Vakgroep Kleine Huisdieren, Faculteit Diergeneeskunde,
Universiteit Gent, Salisburylaan 133, 9820 Merelbeke

Femke.Mortier@UGent.be

SAMENVATTING

De doelstelling van deze studie bestond uit het uitvoeren van een retrospectieve analyse van de transfusies die in het jaar 2018 aan honden werden toegediend in de Kliniek Kleine Huisdieren van de Faculteit Diergeneeskunde in Merelbeke, Universiteit Gent. In totaal werden 87 honden in deze studie ingesloten, die samen een totaal van 140 transfusies hebben gehad. Het aantal transfusies met "packed red blood cells" (n = 85) lag driemaal hoger dan het aantal toedieningen van "fresh frozen plasma" (n = 28) of vers volbloed (n = 27). De meest frequent gestelde diagnose was primaire immuungemedieerde hemolytische anemie en daarnaast lag bij opvallend veel honden, in vergelijking met eerdere studies, coumarine-intoxicatie aan de basis van het uitvoeren van een transfusie met bloedproducten. Transfusiereacties traden op bij 23,2% van de transfusies, waarbij koorts het vaakst werd gezien.

ABSTRACT

The objective of this study was to perform a retrospective analysis of transfusions that were given to dogs in the year 2018 at the Small Animal Clinic of the Faculty of Veterinary Medicine of Ghent University. A total of 87 dogs were included in this study, which together accounted for a total of 140 received transfusions. The number of packed red blood cell transfusions (n = 85) was three times higher than that of fresh frozen plasma (n = 28) or fresh whole blood (n = 27) administrations. Primary immune-mediated hemolytic anemia was diagnosed most frequently and, in comparison with previous studies, anticoagulant rodenticide intoxication was seen in a remarkably high number of dogs receiving blood products. Transfusion reactions were noted in 23.2% of transfusions, with fever occurring most frequently.

INLEIDING

Sinds meerdere decennia worden er in de diergeneeskunde transfusies van bloedproducten uitgevoerd en de frequentie ervan neemt alsmaar toe. Er zijn verschillende zaken waarmee rekening gehouden moet worden. Een eerste parameter is de bloedgroep van zowel donor als acceptor. Aangezien honden geen natuurlijke alloantistoffen bezitten, verloopt de eerste transfusie in principe veilig, ook wanneer de bloedgroep tussen donor en acceptor niet compatibel is (Giger, 2015). Bij de tweede transfusie van bloedproducten kunnen er echter wel reacties voorkomen door het optreden van sensitisatie (Abrams-Ogg et al., 2016). Het belangrijkste bloedgroepsysteem met het oog op transfusiereacties is het "dog erytrocyte antigen" (DEA) 1 en met name het type DEA 1.1, aangezien dit de grootste antigeniciteit vertoont (Davidow, 2013; Giger, 2015). Naast het typeren van de bloedgroep speelt ook een kruisproef ("crossmatch") een belangrijke rol bij transfusies van bloedproducten. Het uitvoeren van een dergelijke kruisproef wordt aanbevolen wanneer het niet duidelijk is of er op een eerder moment bij de acceptor een transfusie van bloedproducten werd uitgevoerd en er tijdens een eerste transfusie een hemolytische reactie is opgetreden (Davidow, 2013). Verder wordt een hond vanaf vier dagen na de vorige transfusie als potentieel gesensiti-

seerd beschouwd en dient vanaf dat moment dus ook een kruisproef te worden uitgevoerd (Abrams-Ogg et al., 2016).

Verschillende bloedproducten kunnen worden toegediend. De eerste categorie is vers volbloed waarin rode bloedcellen, witte bloedcellen, plasmaproteïnen, stabiele en labiele stollingsfactoren (deze laatste zijn fibrinogeen, factor V en factor VIII) aanwezig zijn alsook bloedplaatjes indien het bloed binnen de vier tot zes uur na afname aan de acceptor wordt gegeven (Davidow, 2013; Sullivan et al., 2014). Na het centrifugeren van (vers) volbloed kunnen verscheidene componenten van het bloed apart worden bewaard (Abrams-Ogg et al., 2016). "Packed red blood cells" (pRBC) waarin enkel rode bloedcellen aanwezig zijn, is een eerste component. Een tweede component is "fresh frozen plasma" (FFP). Het bevat alle stollingsfactoren, albumine, antiproteasen, anticoagulantia en immunoglobulines (Davidow, 2013). Daarnaast bestaat er ook "frozen plasma" (FP) waarin geen labiele stollingsfactoren meer aanwezig zijn doordat het meer dan zes uur na afname werd ingevroren of reeds langer dan een jaar werd bewaard. Andere bloedproducten kennen een minder frequente toepassing in de diergeneeskunde (Davidow, 2013; Sullivan et al., 2014).

Bij het uitvoeren van een transfusie van bloedproducten kunnen zich transfusiereacties voordoen. De meest voorkomende zijn acute immunologische transfusiereacties waarbij volgende symptomen kunnen optreden: koorts, sufheid, tremoren, onrust, vocalisatie, tachycardie, aritmieën, hypotensie, tachypnee/dyspnee, speekselen, braken, epileptiforme aanvallen, urticaria/angio-oedeem en hartstilstand (Abrams-Ogg et al., 2016). Deze acute immunologische reacties treden op bij 1-28% van de transfusies, afhankelijk van het gebruikte bloedproduct (minder transfusiereacties bij FFP dan bij pRBC of vers volbloed) en de gehanteerde criteria voor transfusiereacties (Snow et al., 2010; Holowaychuk et al., 2014; Abrams-Ogg et al., 2016).

Het doel van deze retrospectieve studie was om de frequentie na te gaan waarmee transfusies worden uitgevoerd bij honden aan een universitaire dierenkliniek en om de types van gebruikte bloedproducten, de indicaties voor toediening en eventueel optredende transfusiereacties te beschrijven.

MATERIAAL EN METHODEN

Selectiecriteria

In deze retrospectieve studie werd een analyse verricht van de verschillende transfusies van bloedproducten die werden uitgevoerd bij honden aangeboden aan de Kliniek Kleine Huisdieren van de Faculteit Diergeneeskunde van de Universiteit Gent van 1 januari 2018 tot en met 31 december 2018. Deze acceptoren werden geïdentificeerd door middel van het afzonderlijk invoeren van de verschillende bloedproducten als zoekterm in het software-programma. Enkel de honden waarvan een volledig elektronisch dossier voorhanden was en waarbij het papieren formulier met de gegevens over de monitoring van de transfusie en eventuele transfusiereacties aanwezig was (een gestandaardiseerd document dat bij iedere transfusie wordt gebruikt), werden opgenomen in deze studie. Honden die wel een transfusie hadden ondergaan, maar waarvan het dossier niet compleet was en/of het gestandaardiseerde document van de monitoring van de transfusie van bloedproducten niet aanwezig was, werden uitgesloten.

Procedures

Voor elke acceptor-hond werd het signalement (i.e. geslacht, leeftijd en ras) gedocumenteerd evenals de bloedgroep (DEA1.1-negatief of -positief) en werd aangegeven of al dan niet een kruisproef werd uitgevoerd. Vervolgens werden volgende gegevens onderzocht: het type bloedproduct (vers volbloed, pRBC, FFP of FP), de hoeveelheid product per transfusie (in ml/kg) en het totaal aantal transfusies per acceptor. Bijkomend werd er gedocumenteerd wat de indicaties waren voor de transfusie; daarbij werden de volgende categorieën onderscheiden: probleem met de primaire hemostase, probleem met de secundaire hemostase, anemie ten gevolge van een bloeding (door een andere oorzaak dan coagulopathie), anemie ten gevolge van hemolyse, anemie ten gevolge van een andere oorzaak en hypoalbuminemie.

De uiteindelijke diagnose of onderliggende aandoening die had geleid tot coagulopathie, anemie of hypoalbuminemie bij de acceptor werd eveneens gedocumenteerd. Verder werd aandacht besteed aan transfusiereacties (i. e. koorts, tachycardie, tachypnee, aritmieën, hypotensie, braken, urticaria) en andere opmerkingen die in het dossier van de acceptor genoteerd werden. Tot slot werd ook de pre- en posttransfusie-hematocriet (Hct) genoteerd voor transfusies met pRBC en vers volbloed.

Statistische analyse

De gegevens werden geanalyseerd via 'Microsoft Excel 2010' en werden geïnterpreteerd door middel van beschrijvende statistiek, waarbij de minimale, maximale en gemiddelde waarden ± standaarddeviatie (SD) werden berekend.

RESULTATEN

Van 1 januari 2018 tot en met 31 december 2018 werd er bij 118 honden minimaal één transfusie van een bloedproduct uitgevoerd. Deze honden ondergingen in totaal 177 transfusies. Bij 31 acceptoren was het dossier onvolledig of het standaarddocument met de gegevens over de monitoring van de transfusie en

eventuele transfusiereacties afwezig, waardoor deze werden uitgesloten voor verdere analyse. Er werden bijgevolg 87 honden ingesloten in deze studie die samen 140 transfusies hebben ontvangen.

Signalement

In deze studie waren 43/87 honden (49%) mannelijk (20/43 mannelijk en intact en 23/43 mannelijk en gecastreerd). Het aantal teven bedroeg 51% (44/87) met 17/44 vrouwelijk en intact en 27/44 gecastreerde teven. De gemiddelde (±SD) leeftijd was zes (±3,5) jaar met een minimum van vijf maanden en een maximale leeftijd van 14 jaar. Het ras dat het frequentst werd aangeboden was een kruising (12,6%; n=11), gevolgd door de Amerikaanse staffordshire terriër, de Engelse cocker spaniël en de labrador retriever (n=5), die elk 5,7% van het totale aantal honden uitmaakten.

Bloedgroepbepaling van de acceptor

Bij de acceptoren werd enkel de bloedgroep DEA 1.1 getest door middel van een snelle in-huistest (Alvedia® Lab Test DEA 1). Bij 25/87 (28,7%) van de honden werd geen bloedgroepbepaling uitgevoerd. Bij 27/62 (43,5%) van de geteste acceptoren was de bloedgroep DEA 1.1-negatief en 35/62 (56,5%) acceptoren waren DEA 1.1-positief.

Kruisproef

Bij 17/87 honden (19,5%) werd er een kruisproef uitgevoerd. Bij 15/17 honden was de reden dat ze reeds eerder een bloedproduct hadden ontvangen, bij de resterende twee honden omdat het niet bekend was of ze eerder al eens een transfusie hadden ondergaan. Van de 70/87 (80,5%) honden waarbij er geen kruisproef plaatsvond, ondergingen 17/70 (24,3%) acceptoren meerdere transfusies. De tijd die tussen deze transfusies zat, bedroeg telkens slechts een aantal uren tot een maximum van twee dagen.

Toegediende bloedproducten

Bij het bepalen van de frequentie van het gebruik van de bloedproducten werd het toedienen van de combinatie van pRBC en FFP als één type bloedproduct beschouwd. Hierdoor werden bij de 140 transfusies in totaal 125 bloedproducten gebruikt. Het bloedproduct dat het frequentst werd toegediend was pRBC alleen, meer bepaald in 70/125 (56%) gevallen. Bij 27/125 toegediende bloedproducten (21,6%) ging het om vers volbloed en bij 15/125 (12%) om een combinatie van een transfusie met pRBC en één met FFP. Er werd enkel FFP toegediend in 13/125 gevallen (10,4%). Er werd geen enkele transfusie met FP uitgevoerd.

Aantal transfusies en hoeveelheid product per transfusie

Bij 58/87 honden (66,7%) werd er eenmalig een transfusie uitgevoerd met pRBC, FFP, vers volbloed of een combinatie van pRBC en FFP. Daarnaast waren er 22/87 acceptoren (25,3%) die op twee verschillende tijdstippen transfusies kregen, 5/87 honden (5,8%) die drie transfusies ontvingen en 2/87 honden (2,3%) die in totaal vier transfusies kregen toegediend in 2018. Het bloedproduct dat het meest frequent in veelvoud werd toegediend was pRBC. In Tabel 1 wordt de hoeveelheid product weergegeven (in ml/kg) die per transfusie werd toegediend.

Indicaties voor het toedienen bepaalde bloedcomponenten

Voor elk van de 125 toegediende bloedproducten werd de hoofdindicatie gedocumenteerd. Indien een hond zowel FFP als PRBC nodig had omwille van bloedverlies dat was opgetreden als gevolg van coumarine-intoxicatie, DIS of hemofilie A, was de indicatie voor de toediening van de combinatie van beide producten dus een probleem met de secundaire hemostase. In Tabel 2 worden de frequenties weergegeven van de verschillende indicaties om een transfusie uit te voeren evenals de bijhorende onderliggende aandoeningen die werden gediagnosticeerd.

Onderliggende aandoening

Rekening houdend met de verschillende uiteindelijke diagnosen werden volgende onderliggende aandoeningen vastgesteld: het meest voorkomend was immuungemedieerde hemolytische anemie (IMHA) bij 16/87 honden (18,4%) (15 keer primaire IMHA en één keer secundaire IMHA), gevolgd door coumarine-intoxicatie bij 14,9% van de acceptoren (n=13). Een bloedende neoplasie was in 13,8% van de gevallen aanwezig (n=12). In 8/12 van deze gevallen werd de neoplasie histologisch bevestigd (hemangiosarcoom

Tabel 1. Gebruikte producten en toegediende hoeveelheid per transfusie in ml/kg.

	"Packed red blood cells"	"Fresh frozen plasma"	Vers volbloed
Aantal keer toegediend	85	28	27
Gemiddelde ± SD	13 ± 3,8	10,9 ± 3,6	16,4 ± 3,5
Minimum	5	4	9
Maximum	23,3	20	20
Meest frequent toegediend	10 of 15	10	20

Tabel 2. Frequentie van voorkomen van de verschillende indicaties voor het toedienen van een transfusie met "packed red blood cells", "fresh frozen plasma" of vers volbloed en de bijhorende diagnosen.

Indicatie voor de toediening van een bloedproduct	Aantal keer voorgekomen (totaal = 125)	Percentage van alle indicaties	Diagnosen (onderliggende aandoeningen)
Probleem met primaire hemostase	32	25,6 %	Primaire en secundaire ITP, Evans' syndroom, hepatopathie
Probleem met secundaire hemostase	27	21,6 %	Coumarine-intoxicatie, DIS (door sepsis of hitteslag), hepatopathie, hemofilie A, Angiostrongylose
Anemie ten gevolge van een loeding (door een andere oorzaak dan coagulopathie)	29	23,2 %	Bloedende neoplasie, iatrogene bloeding, bloeding door trauma, gastro-intestinale bloeding door NSAIDs, gastro-intestinale bloeding door IBD, epistaxis door Aspergillose, bloeding van onbekende oorsprong
Anemie ten gevolge van hemolyse	31	24,8 %	Primaire en secundaire IMHA, DIS (door anafylactische shock), hemofagocyterende neoplasie, Leishmaniose, Evans' syndroom
Anemie ten gevolge van een andere oorzaak	5	4,0 %	Chronische nierziekte, acute myeloïde leukemie
Hypoalbuminemie	1	0,8 %	Septische peritonitis

DIS: diffuse intravasale stolling; IBD: inflammatory bowel disease; IMHA: immuungemedieerde hemolytische anemie; ITP: immuungemedieerde trombocytopenie; NSAIDs: niet-steroïdale anti-inflammatoire geneesmiddelen

milt, hemangiosarcoom hartoortje, hepatoom lever, leiomyosarcoom vagina, alimentair lymfoom dunne darm). Immuungemedieerde trombocytopenie (ITP) werd bij 11,5% van de honden (n=10) waargenomen (negen primaire ITP, één secundaire ITP), iatrogene bloeding bij 6,9% (n=6) en diffuse intravasale stolling (DIS) bij 5,8% (n=5) (drie als gevolg van bacteriële infectie/sepsis, één door hitteslag en één door anafylactische shock). Anemie door chronische nierziekte kwam bij 3/87 acceptoren voor (3,5%). Hemofagocyterende neoplasie, acute myeloïde leukemie, hemofilie A, hepatopathie door een onbekende oorzaak en een bloeding van onbekende oorsprong werden elk bij 2/87 honden gezien (2,3%). De volgende diagnosen werden elk één keer gesteld (1,2%): bloeding door trauma, gastro-intestinale bloeding ten gevolge van niet-steroïdale anti-inflammatoire geneesmiddelen (NSAIDs), gastro-intestinale bloeding ten gevolge van "inflammatory bowel disease" (IBD), nasale aspergillose met erge epistaxis tot gevolg, angiostrongylose, leishmaniose, Evans' syndroom en hypoalbuminemie ten gevolge van septische peritonitis. Tot slot was er bij 4/87 of 4,6% van de gevallen geen definitieve diagnose bekend.

Transfusiereacties

Ook bij de berekening van de transfusiereacties werd de toediening van de combinatie van pRBC en FFP als één transfusie beschouwd, waardoor het totaal van de transfusies voor de berekening van de transfusiereacties op 125 kwam. Een transfusiereactie trad op bij 29/125 transfusies (23,2%). Bij 14/29 (48,3%) van de transfusiereacties ging het om honden die enkel koorts ontwikkelden. Een hond werd geacht koorts te hebben wanneer de lichaamstemperatuur tijdens de transfusie steeg tot boven de 39°C terwijl deze voor aanvang nog <39°C bedroeg. Bij 7/29 (24,1%) van de transfusiereacties was er enkel sprake van braken. Bij 3/29 transfusiereacties (10,3%) werd er zowel koorts als braken waargenomen. In 2/29 gevallen (6,9%) ging het om een huidreactie en daarnaast waren hypotensie, een combinatie van koorts en tachypnee, en ventriculaire premature contracties (VPC's) die elk 1/29 transfusiereacties (3,5%) uitmaakten.

Indien koorts werd waargenomen, werd de transfusie tijdelijk stopgezet en een antihistaminicum toegediend (diphenhydramine 2 mg/kg PO). Eens de temperatuur opnieuw was genormaliseerd, werd de transfusie terug opgestart aan de helft van de normale snelheid en indien er geen verdere reacties optraden, werd de snelheid na een half uur opnieuw verhoogd. Bij honden die braakten, werd dezelfde procedure gevolgd, maar werd bijkomend ook een anti-emeticum toegediend (maropitant 1mg/kg IV). Zo kon de transfusie toch nog volledig worden toegediend in 22/29 (75,9%) van de gevallen waar een transfusiereactie was opgetreden. De reden voor het stoppen van de transfusie bij de overige acceptoren had in 6/7 gevallen als reden dat er koorts was opgetreden en de lichaamstemperatuur niet normaliseerde na het onderbreken van de transfusie en het toedienen van een antihistaminicum. Voor één acceptor was de reden onbekend.

Pre- en posttransfusie-hematocrietwaarde

Specifiek voor de pRBC-transfusies werd er een gemiddelde pretransfusie-hematocriet (Hct) van 15,9%

± 7,1 gemeten. Het gemiddelde van de posttransfusie Hct was 23,4% ± 8,1. Op basis van de pre- en posttransfusie-Hct kon er een verschil tussen beide berekend worden. In drie gevallen werd er een daling opgemerkt tijdens de pRBC-transfusie, waarbij de sterkste daling 9,3% bedroeg en de posttransfusie-Hct bij die hond 27%. Bij de overige 82 pRBC-transfusies werd er een toename van de Hct gezien. Over alle pRBC-transfusies heen trad er een gemiddelde stijging op van 7,5% ± 6,4.

Bij de versvolbloedtransfusies was de gemiddelde pretransfusie-Hct 19,9% ± 7,9. Het gemiddelde van de posttransfusie-Hct bedroeg 23,3% ± 6,3. Bij vijf transfusies trad er een daling van de Hct op, de overige 22 transfusies leidden tot een stijging van de Hct. Over alle versvolbloedtransfusies heen was er een gemiddelde toename in de Hct van 3,4% ± 4,8.

DISCUSSIE

Hoewel slechts een korte tijdspanne van één jaar werd onderzocht, konden toch 87 honden in deze studie ingesloten worden. Een groot deel van de acceptoren (30/87 of 34,5%) ontving meerdere transfusies, wat de mogelijkheid bood om in deze retrospectieve studie een aanzienlijk aantal van 140 transfusies te onderzoeken. Hierbij werden zowel transfusies met pRBC, FFP als vers volbloed ingesloten.

Wat betreft de bloedgroep blijkt uit deze studie dat bij ongeveer één vierde van de acceptoren geen bloedgroepbepaling was gebeurd alvorens een transfusie toe te dienen. De reden hiervoor was enerzijds dat deze honden nog niet eerder een transfusie ondergaan hadden en dus nog niet gesensitiseerd waren. Anderzijds werd er in het jaar 2018 initieel nog niet standaard getest voor de bloedgroep DEA 1.1 bij de acceptor, aangezien er enkel gebruik werd gemaakt van DEA 1.1-negatieve donoren in die periode. Daardoor kon er dus geen sensitisatie tegen DEA 1.1 optreden. Doordat er regelmatig een tekort aan donoren was, mede door het feit dat elke donor maar om de drie maanden bloed mag geven, werd deze strategie recent aangepast. Nu worden alle acceptoren aan de Kliniek Kleine Huisdieren (Universiteit Gent) getest op het DEA 1.1-antigen, en DEA 1.1-positieve acceptoren ontvangen bloedproducten van een DEA 1.1-positieve donor. Andere bloedgroepen worden niet getest bij donoren of acceptoren, aangezien het DEA 1.1-antigen de grootste antigeniciteit vertoont (Davidow, 2013; Giger, 2015). Iets meer dan de helft van de acceptoren in deze studie was DEA 1.1-positief, wat vergelijkbaar is met hetgeen beschreven werd in eerdere studies (42-71%) (Tocci, 2010; Ergul Ekiz et al., 2011; Ferreira et al., 2011). Dit benadrukt het belang van het insluiten van DEA 1.1-positieve bloeddonoren, teneinde de donorpool voldoende groot te houden.

De meest frequent toegediende hoeveelheid vers volbloed was 20 ml/kg en kwam overeen met de aanbevolen hoeveelheid die in de literatuur wordt beschreven (Sullivan et al., 2014). Wat betreft de transfusie met pRBC en/of FFP wordt in de literatuur een vereiste minimumhoeveelheid van 6-10 ml/kg vermeld (Sullivan et al., 2014). Deze minimumhoeveelheid werd in de voorliggende studie niet bij alle transfusies gehaald als gevolg van de grootte van de acceptor. In de meeste gevallen werd er echter wel voldaan aan de vereiste hoeveelheid.

Wat betreft de onderliggende diagnosen werd er een vergelijking gemaakt met een eerdere retrospectieve studie waarin in 2014 een totaal van 211 honden onderzocht werden die een transfusie met pRBC hadden ontvangen evenals bijkomende bloedproducten indien dat nodig was (Holowaychuk et al., 2014). De volgende diagnosen kwamen in die studie het vaakst voor: IMHA (31,1%), neoplasie (16,1%), ITP (6,2%), trauma (5,2%), en coumarine-intoxicatie (1,9%). De diagnose IMHA werd ook in de huidige studie het meest frequent gediagnosticeerd. Coumarine-intoxicatie kwam in de huidige studie echter veel frequenter voor dan in de studie van Holowaychuk et al. (2014). Mogelijke verklaringen voor dit grote verschil zijn ten eerste dat bij de eerdere studie enkel pRBC-transfusies als uitgangspunt werden beschouwd, waardoor honden die bijvoorbeeld enkel een FFP-transfusie ondergingen, niet ingesloten werden. Een behandeling met FFP of FP kan toereikend zijn bij coumarine-intoxicatie, indien er nog geen sprake is van gedecompenseerde anemie. Daarnaast is het mogelijk dat er regionale verschillen zijn voor wat het gebruik van rattengif betreft, aangezien de andere studie in Canada werd uitgevoerd. Voor wat een bloedende neoplasie betreft werd er een vergelijkbaar percentage aangetroffen.

De transfusiereacties in beschouwing genomen, werd in eerdere onderzoeken een vergelijkbaar tot iets hoger percentage van neveneffecten aangetoond. In de studie van Holowaychuk et al. (2014) wordt een percentage van 28% vermeld. In een recent onderzoek van Maglaras et al. (2017), waarin de toediening van bloedproducten aan 210 acceptoren en een totaal van 333 pRBC- en versvolbloedtransfusies werden geänalyseerd, werd een percentage van 25% aangetoond. Een mogelijke verklaring voor het iets lagere percentage van transfusiereacties in de huidige studie zou het insluiten van FFP-transfusies kunnen zijn, waarbij het risico op immunologische reacties lager ligt. In de studie van Maglaras et al. (2017) werden volgende transfusiereacties gerapporteerd, waarbij bij sommige dieren meer dan een van deze reacties optrad: koorts (48,4% van de transfusiereacties), hemolyse (25%), braken en diarree (13,1%), tachypnee (15,5%), tachycardie/aritmie (14,3%), neurologische symptomen (6%), hypotensie (3,6%) en sterfte/hartstilstand (7,1%) (Maglaras et al., 2017). Koorts werd in die studie (48,4%) net als in de huidige studie (62,1%) het frequentst gezien. Braken vormde in de voorlig-

gende studie een meer dan dubbel zo grote groep van transfusiereacties dan in de studie van Maglaras et al. (2017). Tachypnee en tachycardie werden in de huidige studie dan weer veel minder vaak als neveneffect waargenomen dan in de studie van Maglaras et al. (2017), mogelijk omdat de definities daarvoor tussen beide studies verschilden en de onderzochte honden vaak reeds tachycardie en/of tachypnee vertoonden bij het opstarten van de transfusie als gevolg van anemie. Aritmie in de vorm van VPC's, kwam in de huidige studie eveneens minder vaak voor dan in de studie van Maglaras et al. (2017); de frequentie van aritmieën zou in de huidige studie echter onderschat kunnen zijn, aangezien monitoring door middel van een elektrocardiogram enkel gebeurde indien er tijdens de monitoring onregelmatigheden werden waargenomen bij hartauscultatie. In de huidige studie werd hemolyse niet als neveneffect opgemerkt, terwijl dit 25% van de transfusiereacties uitmaakte in de retrospectieve studie van Maglaras, et al. (2017). Hemolyse werd echter niet standaard op het document van de monitoring van de transfusie van bloedproducten vermeld, omdat dit hoofdzakelijk na en niet tijdens de transfusie wordt opgemerkt, meestal doordat er pigmenturie wordt gezien (door de aanwezigheid van hemoglobine in de urine) of hemolytisch plasma/serum. Daarom kan het optreden van hemolyse als transfusiereactie en bijgevolg ook het totale aantal transfusiereacties onderschat zijn in de huidige studie. Het feit dat hemolyse niet stelselmatig werd gerapporteerd, kan ook verklaren waarom transfusiereacties als koorts en braken in de huidige studie relatief frequenter werden opgemerkt dan in de studie van Maglaras et al. (2017).

In een retrospectieve studie van Silvestrini et al. (2009), waarbij enkel pRBC-transfusies onderzocht werden, werden vergelijkbare waarden aangetoond met die van de voorliggende studie voor wat betreft de minimum-, maximum- en gemiddelde pre- en posttransfusie-Hct-waarden evenals de gemiddelde stijging ervan. Ook de Hct-waarden in de huidige studie bij versvolbloedtransfusies werden vergeleken met de waarden in een eerdere studie, waarbij enkel versvolbloedtransfusies onderzocht werden (Ognean et al., 2015). De hoeveelheid product die werd toegediend in de studie van Ognean et al. (2015) was gemiddeld 7,5 ml/kg, wat lager is dan in de huidige studie (16,4 ml/kg). De stijging van de Hct bedroeg in de studie van Ognean et al. (2015) desondanks gemiddeld 10%, wat drie keer zo hoog is als in de huidige studie. Een mogelijke verklaring hiervoor is dat vers volbloed in de huidige studie voornamelijk werd toegediend aan honden die aan acuut ernstig bloedverlies leden ten gevolge van trombocytopenie, waardoor ze nog verder bleven bloeden tijdens (een deel van) de transfusie. In de studie van Ognean et al. (2015) waren de voornaamste indicaties voor de transfusie met vers volbloed geen acute bloeding maar anemie door lymfoom of nierziekte. Het feit dat de stijging van de Hct-waarde in de huidige studie dubbel zo groot was na de toediening van pRBC dan van vers volbloed, kan dan op zijn beurt worden verklaard door het feit dat de gemiddelde toegediende hoeveelheid bloedproduct ongeveer gelijk was (13 versus 16,5 ml/kg), terwijl vers volbloed slechts voor ongeveer 40% uit rode bloedcellen bestaat.

Ondanks de gemiddelde toename van de Hct na transfusie werd er bij 5/27 versvolbloedtransfusies en 3/85 pRBC-transfusies een daling van de Hct-waarde waargenomen. Deze daling kan enerzijds te wijten zijn geweest aan een actieve bloeding of hemolyse die aanwezig was ten tijde van de transfusie; anderzijds waren de acceptoren bij opname mogelijk hypovolemisch door acuut bloedverlies, wat tot een overschatting van de initiële Hct-waarde heeft geleid.

Zowel bij de pRBC- als versvolbloedtransfusies werd er bij één acceptor een transfusie uitgevoerd ondanks een normale Hctwaarde. In het geval van de pRBC-transfusie ging het om een hond met een ernstige acute iatrogene bloeding. De versvolbloedtransfusie werd toegediend aan een hond met primaire ITP die een invasieve procedure moest ondergaan. Preoperatief werd een transfusie uitgevoerd met vers volbloed, dat trombocyten bevat om het bloedingsrisico tijdens de ingreep te beperken.

De voorliggende studie bevat echter een aantal beperkingen. Het gaat om een retrospectieve studie, waardoor enerzijds meerdere honden uitgesloten moesten worden en anderzijds niet alle gegevens op een gestandaardiseerde manier in de dossiers werden bijgehouden. Een belangrijk gevolg hiervan is dat bepaalde transfusiereacties, zoals hemolyse, mogelijk gemist werden. De gegevens van de donoren, zoals de Hct-waarde, werden evenmin in beschouwing genomen. Bovendien werden de exacte gegevens betreffende de bewaring van de bloedproducten niet opgenomen in deze studie, waardoor een eventuele vermindering van de kwaliteit van het product door bewaring niet kon worden onderzocht.

CONCLUSIE

De toediening van bloedproducten is een frequente procedure in de Kliniek Kleine Huisdieren van de Faculteit Diergeneeskunde van de UGent. In totaal werden 87 honden in deze studie ingesloten, die samen een totaal van 140 transfusies hebben gehad. Wat betreft de bloedgroep werd er een gelijkaardige frequentie van 56,5% DEA 1.1-positieve acceptoren als in de literatuur (42-71%) waargenomen, wat het belang van het insluiten van DEA 1.1-positieve donoren benadrukt. "Packed red blood cells" werden het vaakst toegediend en een probleem met de secundaire hemostase was de meest voorkomende indicatie voor het geven van bloedproducten, gevolgd door een probleem met de primaire hemostase en hemolytische anemie. De diagnose die net als in een eerdere retrospectieve studie het meest frequent gesteld werd,

was IMHA; daarnaast was coumarine-intoxicatie echter een opvallend frequent voorkomende diagnose. Verder kwam het percentage van transfusiereacties overeen met eerdere bevindingen, waarbij koorts het vaakst optrad. Transfusiereacties leidden slechts bij 7/140 transfusies tot een permanente stopzetting van de transfusie. De gemiddelde stijging van de Hct bij het uitvoeren van een pRBC-transfusie was ruim twee keer zo groot als bij een versvolbloedtransfusie, waarbij de gemiddelde toename van de Hct-waarde bij versvolbloedtransfusies minder dan de helft bedroeg dan in eerdere retrospectieve studies.

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Epiglottic retroversion in nine dogs

Epiglottisretroversie bij negen honden

K. Van Ginneken, B. Van Goethem, N. Devriendt, T. Bosmans, H. de Rooster

Vakgroep Kleine huisdieren, Faculteit Diergeneeskunde, Universiteit Gent,
Salisburylaan 133, B-9820 Merelbeke, België

katrijn_van_ginneken@hotmail.com

ABSTRACT

Epiglottic retroversion (ER) is an uncommon and poorly understood disorder of the upper respiratory tract in small breed dogs. In this retrospective study, perioperative characteristics, surgical technique, outcome, and complications in nine dogs that underwent surgical treatment for ER and/or concurrent upper respiratory tract disorders, were evaluated. The most frequently reported clinical symptoms were chronic intermittent inspiratory stridor (89%), exercise intolerance (78%), and dyspnea (67%). Concurrent respiratory disorders were highly prevalent (78%). Five dogs initially underwent a temporary epiglottopexy and two a permanent epiglottopexy. In two dogs, both suffering from concurrent laryngeal paralysis, only a unilateral cricoarytenoid lateralization was performed. After initial clinical improvement, temporary and permanent epiglottopexy eventually failed in 4/6 dogs (67%) that were available for follow-up, necessitating partial epiglottectomy as revision surgery. This resulted in a successful long-term outcome in 5/6 of these dogs (83%). In the dogs with primary ER or in cases where the presence of secondary ER led to significant respiratory symptoms, partial epiglottectomy as a primary surgical technique appeared to be a more permanent treatment option than epiglottopexy. Both dogs with surgically corrected concurrent laryngeal paralysis without epiglottopexy or epiglottectomy showed clinical improvement. This might indicate that, in case of secondary ER, positive results can be achieved after management of the underlying respiratory disorder.

SAMENVATTING

Epiglottisretroversie (ER) is een weinig voorkomende aandoening van de bovenste luchtwegen bij kleine hondenrassen waarover nog weinig bekend is. In deze retrospectieve studie werden de perioperatieve kenmerken, de chirurgische techniek, de resultaten en de complicaties bij negen honden, behandeld voor epiglottisretroversie en/of gelijktijdig voorkomende respiratoire aandoeningen, geëvalueerd. De meest voorkomende symptomen waren chronisch intermitterende inspiratoire stridor (89%), inspanningsintolerantie (78%) en dyspneu (67%). Respiratoire comorbiditeiten waren veelvoorkomend (78%). Bij vijf honden werd een tijdelijke epiglottopexie uitgevoerd en bij twee honden een permanente. Bij twee honden, beide met larynxparalyse, werd enkel een unilaterale crico-arytenoid lateralisatie uitgevoerd. Na initiële klinische verbetering bleek de tijdelijke of permanente epiglottopexie bij 4/6 honden (67%) gefaald te zijn tijdens de follow-up. Daarop werd een partiële epiglottectomie uitgevoerd. Dit resulteerde in een klinische verbetering bij 5/6 honden (83%). Bij een primaire ER of wanneer de aanwezigheid van secundaire ER leidde tot significante ademhalingssymptomen, leek het uitvoeren van een partiële epiglottectomie als primaire chirurgische techniek daarom de meest succesvolle optie. Beide honden met chirurgisch gecorrigeerde larynxparalyse, die geen epiglottopexie of partiële epiglottectomie ondergingen, vertoonden een klinische verbetering. In het geval van secundaire ER kan er mogelijk ook een goed resultaat worden bereikt na behandeling van enkel de onderliggende respiratoire aandoening.

INTRODUCTION

Epiglottic retroversion (ER) is a rare disorder in dogs characterized by retroflexion of the epiglottis towards the rima glottidis during inspiration (Mullins et al., 2014). This leads to an intermittent obstruction of the upper respiratory tract resulting in inspiratory stridor and dyspnea (Skerret et al., 2015). Similar conditions can be found in horses and humans (Woo, 1992; Parente et al., 1998; Lane et al., 2010; Terrón-Canedo and Franklin, 2013). The etiology of ER in dogs is still unknown (Skerret et al., 2015). In previous studies, it has been hypothesized that potential etiologies could be disorders of the epiglottic cartilage (Flanders and Thompson, 2009), hyoepiglotticus muscle (Amis et al., 1996a; Amis et al., 1996b; Flanders and Thompson, 2009), hypoglossal nerves (Holcombe et al., 1997), or hypothyroidism-associated peripheral neuropathy (Panciera, 2001; Cuddon, 2002; Flanders and Thompson, 2009). ER could also be secondary to, or even a component of, other concurrent upper airway disorders, which cause increased turbulence, upper airway resistance, and negative upper airway pressures (Skerret et al., 2015).

In previous studies, surgical management of ER by performing a temporary or permanent epiglottopexy has been described (Skerret et al., 2015). However, the occurrence of epiglottopexy failure was high with 37% of temporary and 62% of permanent epiglottopexies failing (Skerret et al., 2015). Partial epiglottectomy has been performed as a revision technique in two previous cases and has shown promising results (Mullins et al., 2014; Skerret et al., 2015).

The aim of this retrospective study was to evaluate the signalment, the clinical signs and comorbidities, the laryngoscopic findings, the surgical techniques performed, and the outcome in dogs diagnosed with ER. Based on the current literature regarding ER, the authors hypothesize that respiratory tract disorders and neurological comorbidities are highly prevalent in dogs with ER and that the complication rate and results during follow-up depend on the type of surgical intervention and the presence of comorbidities.

MATERIALS AND METHODS

Dogs diagnosed with ER at the Small Animal Teaching Hospital of the Faculty of Veterinary Medicine, Ghent University between 2017 and 2019 were included in this study. ER was diagnosed during (video-)laryngoscopy after physical examination. The diagnosis of ER was confirmed when the epiglottis was not positioned against the base of the tongue at inspiration. The dogs in this study were classified as low or high grade based on the laryngoscopic assessment of the severity of the obstruction of the rima glottidis and the presence of structural abnormalities of the epiglottis, by different surgeons. In low-grade patients, the epiglottis was elevated from the tongue base throughout the respiratory cycle, without showing any ventral movement during inspiration, resulting in a partial obstruction of the rima glottidis. In high-grade patients, the epiglottis retroflexed caudally on inspiration, resulting in complete obstruction of the rima glottidis; in some cases, its tip was even pulled into the rima glottidis.

Additionally, findings on complete blood analysis, thoracic radiographs, tracheoscopy, bronchoscopy, and/or electrophysiological examination were reviewed retrospectively.

All of the dogs underwent at least one surgical procedure, either to correct ER and the (potential) accompanying comorbidities, or to correct the assumed underlying respiratory disorder. The initial surgical techniques used to treat ER were either a temporary or a permanent epiglottopexy. The technique for temporary epiglottopexy consisted of placing one or two mattress sutures using polypropylene (Prolene, Ethicon; range, 5/0 to 2/0) between the epiglottis, engaging the epiglottic cartilage, and the glossopharyngeal mucosa at the base of the tongue, as previously described by Flanders and Thompson (2009). To perform a permanent epiglottopexy, a wedge of mucosa ventrorostrally on the epiglottis and another caudally at the base of the tongue were excised. The edges of the wound bed between the glossopharyngeal mucosa and the epiglottis were apposed with a continuous suture line, also engaging the epiglottic cartilage, with 5/0 poliglecaprone (Monocryl, Ethicon) or 3/0 polyamid (Ethilon, Ethicon), after which the position of the epiglottis was assessed with the tongue in a neutral position (Flanders and Thompson, 2009). In case of failure of these techniques, a partial epiglottectomy was performed by excising one third to two thirds of the distal epiglottis, followed by an evaluation of the patency of the larynx (Mullins et al., 2014). A cruciate suture was placed to prevent retraction of the mucosae.

The anesthetic and analgesic protocols used were based on the preference of the attending anesthesiologist. All dogs were intubated and anesthesia was maintained using gas anesthesia.

Improvement at follow-up was defined as excellent, good, or moderate based on the decrease in number and severity of the respiratory signs. In case of epiglottopexy failure or no improvement of the symptoms, the outcome was described as bad. Telephone interviews were conducted to provide an extra indication of long-term results. The owners were additionally asked to compare the severity of the respiratory signs prior to treatment (improved, similar, or worse). The owners were also asked if they had noticed any evolution of the disease after treatment (stable, improved, worsened) and if they were satisfied with the result (yes, no).

RESULTS

Patient population

Nine dogs with ER were identified in the hospital's

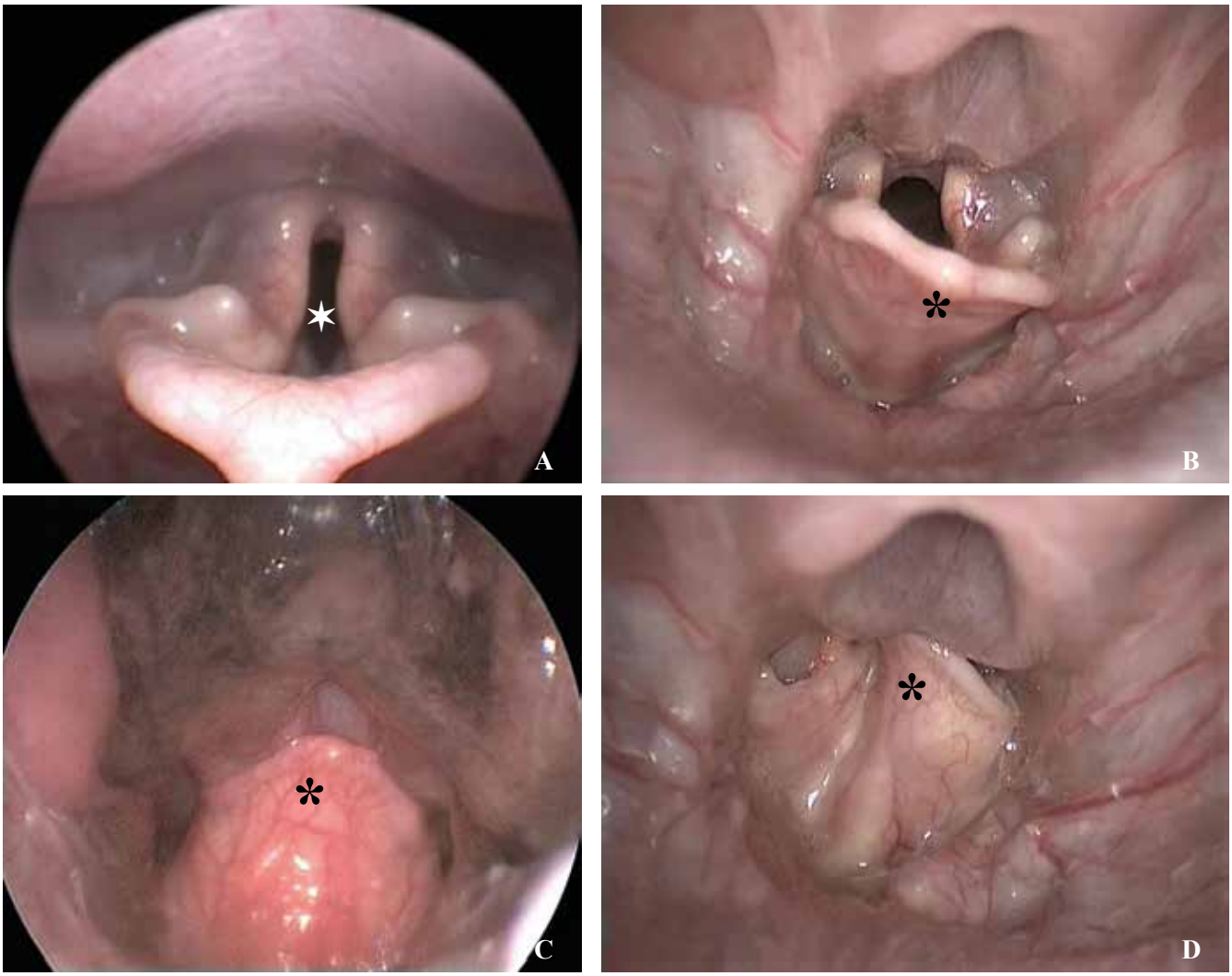

Figure 1. Endoscopic images of the larynx during inspiration. A. Normal larynx with epiglottis (asterisk) positioned against the tongue base, B. low-grade epiglottic reversion (ER) with the epiglottis elevated from the tongue base, C. high-grade ER with the epiglottis closing off the rima glottidis (star), D. high-grade ER with the epiglottis collapsing into the rima glottidis.

patient records during the study's period. Represented breeds were Maltese (n=2), Chihuahua (n=2), Cavalier King Charles spaniel (n=2), Shih tzu (n=1), Yorkshire terrier (n=1), and Pomeranian (n=1). The median age was 8.1 years (range, 1.2 to 9.5 years). Five dogs (56%) were more than seven years old at the time of diagnosis, two dogs (22%) were between three and seven years of age, and two dogs (22%) were less than three years old. Sex distribution was 6/9 male (67%), of which 4/6 were neutered (67%), and 3/9 were female dogs (33%), all spayed. The median weight was 4.6 kg (range, 2.9 to 13.5 kg). The median body condition score was 5/9 (range, 4/9 to 7/9).

Clinical presentation

Eight patients (89%) came in through consultations, whereas one patient (11%) was presented at the emergency services. The presenting respiratory clinical signs were inspiratory stridor (89%), exercise intolerance (78%), dyspnea (67%), coughing and gagging (44%), reverse sneezing (44%), cyanosis (44%), tachypnea (33%), sneezing (22%), and nasal stridor (22%). Six out of the nine dogs (67%) showed intermittent respiratory signs, mainly provoked by excitation or exercise, whereas three dogs (33%) continuously had symptoms. The median time since the start of the symptoms at presentation was twelve months (range, 1 week to 8.6 years).

Diagnostic work-up

All patients underwent laryngoscopic examination (Figure 1). Thirty-three percent of the dogs were considered low grade (3/9). In one of these dogs, the epiglottis was bent caudally with a concave lingual side. Sixty-seven percent were considered high grade (6/9). In one of these dogs, the epiglottis was partially pulled into the rima glottidis during inspiration.

Seven out of the nine patients (78%) had concurrent upper airway disorders (Table 1). On laryngeal inspection, 5/9 dogs (56%) had brachycephalic obstructive

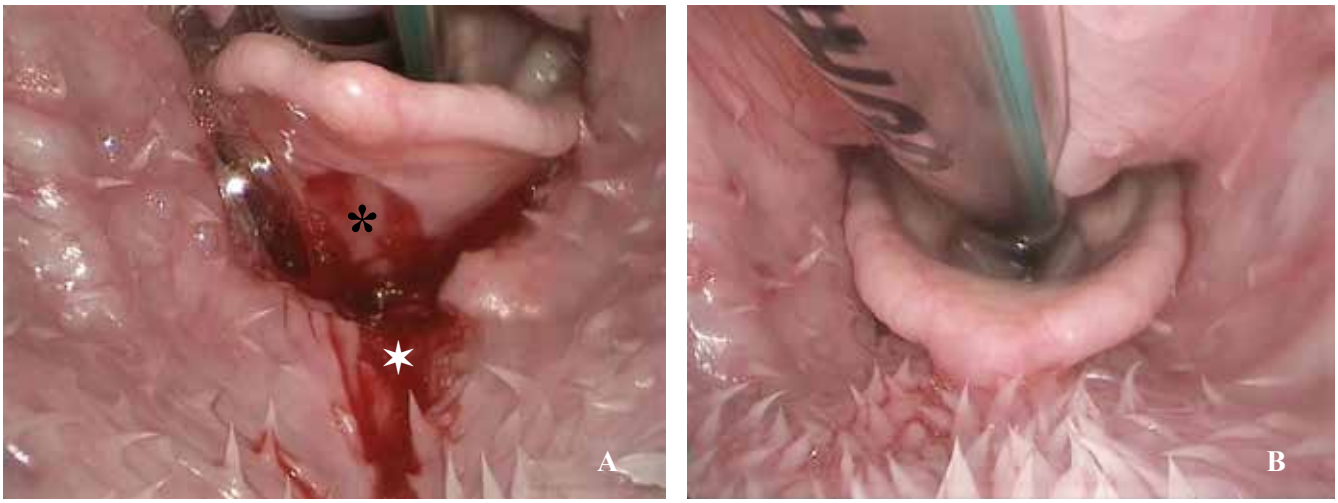

Figure 2. Intra-operative images of a permanent epiglottopexy. A. A wedge of mucosa ventrorostrally on the epiglottis (asterisk) and caudally at the base of the tongue (star) is excised. B. Following fixation by placement of a continuous suture line to the wound bed between the glossopharyngeal and epiglottic mucosae, the position of the epiglottis is assessed with the tongue in a neutral position; the rima glottidis is now patent throughout the respiratory cycle.

airway syndrome (BOAS) and 3/9 dogs (33%) had laryngeal paralysis. The five dogs presented with BOAS had hyperplastic and/or elongated soft palate (4/5), hyperplastic and/or everted tonsils (4/5), laryngeal collapse grade I (1/5), and relative macroglossia (1/5).

Six out of the nine dogs (67%) underwent tracheoscopy of which 2/6 were diagnosed with grade III tracheal collapse, 1/6 with grade I tracheal collapse, 1/6 showed an increased presence of mucus, and 2/6 had no abnormalities. All dogs diagnosed with BOAS had high-grade ER, so did 2/3 dogs with laryngeal paralysis. One of the dogs with grade III tracheal collapse had high-grade ER.

In eight out of the nine dogs (89%) thoracic radiographs were taken prior to surgery. In seven of these patients, no abnormalities of the respiratory tract were detected. In only 1/3 patients diagnosed with tracheal collapse, there were radiological indications present for this disorder. One patient with ER and concurrent laryngeal paralysis underwent electromyographic and electroneurographic examination to screen for potential underlying polyneuropathy, but for both examinations, the results were negative.

Surgical treatment

Temporary epiglottopexy was performed in 5/9 dogs (56%). In one of these five patients also pala-

Table 1. Long-term results (obtained by telephone interview) and epiglottopexy failure in function of the surgical technique performed and the presence of concurrent respiratory tract disorders.

N°	Breed	ER grade	BOAS	LP	TC	Surgical technique	Recurrence	Revision partial epiglottectomy	Long-term result
1	Pomeranian	Low				Temporary EP	Yes	Yes	Moderate
2	Maltese	High	Yes	Yes	Yes	Temporary EP + CAL	Yes	No	Bad
3	Chihuahua	Low			Yes	Temporary EP			Moderate
4	Yorkshire terrier	High	Yes			Temporary EP	Yes	Yes	Good
5	CKCS	High	Yes			Temporary EP + palatoplasty + tonsillectomy			Excellent
6	Shih Tzu	High				Permanent EP	Yes	Yes	Excellent
7	CKCS	High	Yes	Yes		CAL			Excellent
8	Chihuahua	High	Yes			Permanent EP + palatoplasty	Patient lost to follow-up		
9	Maltese	Low		Yes	Yes	CAL			Good

CKCS: Cavalier King Charles spaniel, BOAS: brachycephalic obstructive airway syndrome, LP: laryngeal paralysis, TC: tracheal collapse, EP: epiglottopexy, CAL: unilateral cricoarytenoid lateralisation.

toplasty and unilateral tonsillectomy were performed to treat the concurrent BOAS. One other patient (1/5) additionally underwent cricoarytenoid lateralization for concurrent laryngeal paralysis.

Permanent epiglottopexy was performed in 2/9 dogs (22%) (Figure 2). One of these two patients also underwent concomitant palatoplasty.

All three patients diagnosed with laryngeal paralysis underwent unilateral cricoarytenoid lateralization. For two of these patients (67%), this was the only surgical procedure that was performed. In the remaining patient, temporary epiglottopexy was also performed. The three patients with concurrent tracheal collapse were surgically treated for ER by performing temporary epiglottopexy (n=2), and/or concurrent laryngeal paralysis (n=2).

Short-term evaluation

Six out of the seven patients that underwent epiglottopexy (five temporary and one permanent) were presented for a control visit one month post-operatively. One patient was lost to follow-up. Clinical improvement was seen in 4/6 of these dogs (67%, four temporary and zero permanent), of which one dog that received temporary epiglottopexy, did not show clinical signs anymore. Complications after epiglottopexy occurred in 2/6 dogs (33%, both after temporary epiglottopexy) and consisted of dysphagia, which only lasted for two weeks in one of the dogs. However, none of the patients developed symptoms of aspiration pneumonia. The two patients that only underwent laryngeal paralysis treatment showed major clinical improvement.

Long-term evaluation

Eight patients were available for long-term follow-up. The median long-term follow-up was 16 months (range, 5 to 23 months). Only one patient, that underwent temporary epiglottopexy, was presented at the control visit one year post-operatively. Telephone interviews were available for 8/9 dogs (89%).

One patient that received permanent epiglottopexy, was lost to follow-up. Four out of the six remaining patients (67%) that received epiglottopexy (three temporary, one permanent) showed recurrence of their respiratory symptoms after a period of initial improvement, suggesting failure of the epiglottopexy. The median interval between epiglottopexy and failure of this technique was 1.5 months (range, 1 to 12 months). In three dogs, recurrence of the clinical signs was reported at the time of the control visits (one temporary and one permanent one month post-operatively; one temporary one year post-operatively) and for one, it was the reason for early revisit at 1.5 months post-operatively. Two of these four dogs did not have any comorbidities. One out of two dogs with low-grade ER (50%) and 3/4 dogs with high-grade ER (75%) experienced epiglottopexy failure. Failure of the epiglottopexy was diagnosed with a laryngeal inspection in all four dogs. In 3/4 dogs, partial epiglottectomy was performed as a revision surgery. The other dog initially showed clinical improvement after temporary epiglottopexy and cricoarytenoid lateralization. However, the one-year-post-operative control visit revealed an increase in severity of the respiratory symptoms due to epiglottopexy failure, the limited effect of cricoarytenoid lateralization, and grade III tracheal collapse. No revision surgery was performed in this patient. All dogs that underwent partial epiglottectomy during revision surgery showed clinical improvement. One dog even obtained excellent results and had resolution of all clinical signs. However, two dogs showed dysphagia since the partial epiglottectomy (2/3 dogs or 67%). None of these patients developed clinical signs of aspiration pneumonia within the follow-up period.

Improvement of respiratory signs was present in 5/6 dogs (83%) that underwent temporary or permanent epiglottopexy, or epiglottectomy. One of these dogs only underwent temporary epiglottopexy and palatoplasty for concurrent BOAS, and obtained excellent results remaining free of clinical signs.

Of both dogs that only had their concurrent laryngeal paralysis treated, one clinically improved and one obtained excellent results and remained free of clinical signs. Regarding the patients with concurrent tracheal collapse, the patient with grade I tracheal collapse showed long-term clinical improvement after temporary epiglottopexy. The two patients with medically-treated grade III tracheal collapse were clinically stable.

Eight out of the nine owners participated in the follow-up by telephone survey. The owners hereby described the severity of the respiratory symptoms in comparison to the pre-operative clinical signs as ‘improved’ in six cases (75%), as ‘similar’ in one case (12.5%) and as ‘worse’ in another case (12.5%). The post-operative clinical evolution of the respiratory symptoms was described as ‘stable’ (75%), ‘improving’ (12.5%) and ‘worsening’ (12.5%). Seven out of the eight owners (88%) were satisfied with the treatment of their dog. .

DISCUSSION

Surgical treatment of ER or its underlying respiratory pathology resulted in clinical improvement and high overall owner satisfaction.

More than two thirds of the patients in this study had concurrent respiratory disorders, including BOAS, laryngeal paralysis, or tracheal collapse, at the time of the diagnosis of ER. This complies with the results of Skerret et al. (2015). It is therefore difficult to ascertain the true importance of ER as a primary condition and its ability to cause respiratory discom-

fort on itself. When ER occurs as a secondary disease due to increased inspiratory airway resistance from an underlying primary respiratory disorder, correction of this primary disorder might give clinical improvement of the respiratory issues. In this study, this was demonstrated in two dogs with ER in combination with laryngeal paralysis. Despite limiting surgical treatment to the correction of laryngeal paralysis, major improvement of the clinical signs occurred. In case of ER as a primary pathology, resulting in secondary changes to the upper respiratory tract, surgical correction of ER itself is advised. Only in two dogs, ER was identified as the primary cause of the respiratory symptoms. In most other dogs, the relation between the different diseases was more difficult to unravel, leading to a combination of treatment procedures. This has also been supported by the findings of Skerret et al. (2015), who reported a higher percentage of dogs that showed improvement of respiratory symptoms after combined treatment of concurrent respiratory tract disorders than of dogs that only underwent surgical treatment for ER.

Although ER can easily be diagnosed via direct laryngoscopy, laryngeal inspection is not always conclusive when the ER only occurs when induced by exercise or excitation (Mullins et al., 2014). Also, any pressure at the level of the epiglottis or rostral lingual traction may result in false negative results (Skerret et al., 2015). Lastly, ER is a relatively rare and recently discovered condition and therefore not widely recognized in veterinary medicine. Therefore, ER might be underdiagnosed and possibly undertreated.

Skerret et al. (2015) described temporary or permanent epiglottopexy for surgical treatment of ER. In the present study, however, almost half of the epiglottopexies failed within two months after the surgery. In the study by Skerret et al. (2015), 37% of the temporary and 62% of the permanent epiglottopexies failed, suggesting that the additional trauma caused by excision of mucosa to obtain a permanent epiglottopexy is unnecessary (Skerret et al., 2015). In the present study, this suggestion could not be enforced due to the small patient population. The epiglottopexy procedures failed in 2/4 patients with concurrent respiratory disorders and both patients without concurrent respiratory disorders. This finding is somehow surprising since, due to the presence of increased negative upper respiratory pressures, a higher prevalence of epiglottopexy failure was expected in patients with concurrent respiratory tract disorders. Furthermore, the grade of ER seemed to affect epiglottopexy failure rates with high-grade dogs showing a higher failure percentage.

Dogs, in which the epiglottopexy seemed to have failed, underwent partial epiglottectomy, which resulted in an overall 83% long-term successful outcome. Removal of the distal tip of the epiglottis avoids complete rima glottidis obstruction when the epiglottis aberrantly retracts on inspiration (Mullins et al., 2014). The potential disadvantage, however, is that fluid or food particles may enter the trachea during swallowing. On the other hand, the importance of the presence of the epiglottis during swallowing is controversial (Medda et al., 2003). In this study, dysphagia was seen in 4/7 dogs, with a higher prevalence after partial epiglottectomy than after epiglottopexy. In one dog, this was only a temporary complication, whereas in the other three dogs, this complication persisted as a mild hindrance. None of these dogs developed aspiration pneumonia within the follow-up period. Further studies are needed to determine the most suited amount of epiglottis to be removed for ER.

The limitations of this study include the retrospective design and small patient population. Therefore, it is impossible to draw any statistical conclusions and larger studies are needed to further define the most successful treatment option for ER. Also, the low owner compliance regarding control visits led to more subjective long-term follow-up data. Furthermore, the surgical interventions in this study were performed by different surgeons, using different suture materials and numbers of sutures. Therefore, it is hard to evaluate the factors influencing the failure rate of the epiglottopexies. Moreover, not all dogs diagnosed with BOAS underwent surgical treatment for this condition, due to owner consent and/or the deemed necessity for surgical treatment.

Since epiglottopexy failure is highly prevalent, performing a partial epiglottectomy as a primary surgical technique appears to be a more satisfying treatment option in case of primary ER or when the presence of secondary ER leads to significant respiratory symptoms. On the other hand, too little is known about the potential risk factors for dysphagia. The high prevalence of concurrent respiratory disorders might indicate that ER is secondary to, or an unrecognized component of, these disorders. Therefore, in selected dogs, satisfying results could possibly also be achieved after management of only the concurrent respiratory disorders. The prognosis after surgical treatment of ER and/or concurrent respiratory disorders is generally favorable.

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Spoorwegen aanleggen om runderpest te bestrijden

Medio negentiende eeuw, lang nadat runderpest in West-Europese landen uitgeroeid was, bleef het grote Rusland kampen met deze verwoestende ziekte, waarvan men de virale oorzaak niet kende, maar terdege wist dat ze besmettelijk was. Vandaar uit verspreidde de ziekte zich regelmatig naar de Oostenrijkse en Pruisische gebieden. Oorlogen verhevigden dit sterk. In 1845 werd de Deen H.C. With, samen met twee Duitsers en een lokale arts ingehuurd om uit te vissen hoe dat kon. With stelde een uitvoerig rapport op waarin hij, naast de runderpest onder meer de uiterst penibele reisomstandigheden beschrijft van het gezelschap, samen met allerlei wetenswaardigheden over de Russische rurale economie en samenleving.

De onderzoekers kwamen tot de bevinding dat de ziekte endemisch was in Oekraïne en in andere zuidelijke gebieden gedomineerd door Rusland, in de steppen rond de Kaspische Zee, etc. Niettemin konden in het goede seizoen van daaruit jaarlijks overschotten onder de vorm van levende dieren naar westelijk en noordelijk Rusland gedreven worden. With beschrijft de afgrijselijke omstandigheden waarin het veedrijven gebeurde, de verzwakking van de dieren en de wrede slachtgewoonten. Bij de grote steden aangekomen, konden de dieren - de overlevenden! - gevoed worden met spoeling van de talrijke wodkastokerijen om te sterken en zelfs vet aan te zetten. Dat laatste was belangrijk, want de in stukken gesneden kwartieren werden afgekookt om het in die streken kostbare vet te gebruiken.

Het spreekt vanzelf dat die jaarlijkse trek de verspreiding van de runderpest bevorderde. Belangrijker nog voor het in standhouden van de ziekte waren de vele ossenkarren die voortdurend allerlei goederen (en mensen!) over soms lange afstanden vervoerden. Vooral de talloze besmette trekossen droegen er toe bij het virus voortdurend ‘uit te voeren’. Het onderzoeksploegje kwam dan ook tot het besluit dat de aanleg van de toen nog niet zo lang geïntroduceerde spoorwegen voor goederenvervoer een oplossing zou bieden. De ossenkarren zouden verdwijnen. Daar kwam nog bij dat de per spoor vervoerde slachtdieren niet zo erg uitgeput werden en beter weerstand zouden bieden tegen de ziekte.

Naar de Deense uitgave in het Nederlands samengevat door Jons Straatman. In: *Argos* (2019) nr. 60, 415-421.

Luc Devriese

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A perirenal fibrosarcoma in a newborn calf

Een perirenaal fibrosarcoom bij een pasgeboren kalf

[1]K. Rosiers, [2]F. Smeets, [2]L. Delooz, [1]V. Saey, [1]M. Vanrobaeys, [3]R. Ducatelle, [1]H. Versnaeyen

[1]Dierengezondheidszorg Vlaanderen, Industrielaan 29, B-8820 Torhout
[2]Association Régionale de Santé et d'Identification Animales, Allée Des Artisans 2, B-5590 Ciney
[3]Department of Pathology, bacteriology and Poultry Diseases, Faculty of Veterinary Medecine, Ghent University, Salisburylaan 133, B-9820 Merelbeke

katrijn.rosiers@dgz.be

ABSTRACT

Congenital tumors are rare in cattle. In this case report, a calf with a congenital perirenal fibrosarcoma is described. A newborn Belgian Blue calf that succumbed shortly after caesarian section was submitted for necropsy at the diagnostic lab of the ARSIA (Association Régionale de Santé et d'Identification Animales). At necropsy, a hemorrhagic firm mass was found surrounding the left kidney. Histopathological examination of the mass revealed a neoplastic cell population. Additional immunohistochemical stainings were performed to identify the tumor. The majority of neoplastic cells stained positive for vimentin but were negative for neurofilaments (NFs), desmin, CD3, CD20, Von Willebrand factor and cytokeratin, indicating a mesenchymal origin. The tumor was diagnosed as a fibrosarcoma. To the authors' knowledge, this is the first case of a congenital perirenal fibrosarcoma reported in a Belgian Blue calf in Belgium.

SAMENVATTING

Congenitale tumoren zijn zeldzaam bij het rund. In dit artikel wordt een kalf met een congenitaal perirenaal fibrosarcoom beschreven. Een pasgeboren Belgisch witblauwkalf stierf kort na de keizersnede en werd aangeboden voor autopsie bij het diagnostisch labo ARSIA (Association Régionale de Santé et d'Identification Animales). Tijdens de autopsie werd er een hemorragische, vast aanvoelende, perirenale massa gevonden. Histopathologisch onderzoek van de massa toonde tumorale cellen aan. Bijkomend immunohistochemisch onderzoek werd uitgevoerd voor verdere identificatie van de tumor. De tumorale massa kleurde positief voor vimentine, maar bleek negatief voor neurofilament (NFs), desmine, CD3, CD20, Von Willebrand-factor en cytokeratine. Op basis van deze bevindingen werd de tumor getypeerd als zijnde van mesenchymale oorsprong, meer bepaald als een fibrosarcoom. Volgens de auteurs is dit het eerste beschreven geval van congenitaal perirenaal fibrosarcoom vastgesteld bij een Belgisch witblauw-kalf in België.

INTRODUCTION

Fibrosarcomas are unusual in cattle, horses and pigs but are more commonly reported in dogs and cats. These malignant tumors of fibrous tissue mostly originate in soft tissue and can invade adjacent bone (Basheer et al., 2014; Britt et al., 1998). Congenital tumors in calves are uncommon (Sickinger et al.,2009, Moore, 2013, Misdorp, 2002a,b).

In calves, spontaneous tumors are referred to as congenital when they appear in fetuses, newborn and very young calves that are less than two months of age, (Turan Yaman et al., 2019; Misdorp, 2002a). When the tumors are present between two and twelve months of age, they are defined as tumors of the juvenile type (Misdorp, 2002a). Most cases turn out to be of mesenchymal origin and four main groups can be distinguished: malignant lymphomas, mesotheliomas,

embryonic tumors (medulloblastoma, nephroblastoma) and hamartomas (Misdorp, 2002b). These tumors all occur sporadically with the exception of malignant lymphoma in twin calves and medulloblastoma. Nephroblastomas in neonatal calves are not rare and often attain a large size (Kirkbride and Bicknell, 1972; Misdorp, 1965). Tumors in the same region but without the epithelial component have been histologically typed as mixed mesodermal tumors (Misdorp, 1965) or fibrochondrolipoma (Donnely et al., 1975). Carcinoma is the most frequent tumor in adult cattle and humans but in calves and in children, carcinomas are virtually absent in the neonatal period (Moore et al., 2013; Misdorp, 2002a). Although rare, in cattle, the majority of fibrous tumors are diagnosed as fibroma or fibrosarcoma (Mc Entee and Nielsen, 1976; Takai et al., 2004; Michishita et al., 2016; Mohana et al., 2016). Congenital fibrosarcoma is rare (Misdorp, 2002b). In this report, the pathological, histological and immunohistochemical examination of a newborn Belgian Blue calf with a congenital fibrosarcoma is described.

CASE DESCRIPTION

Case history and diagnostic protocol

A female Belgian Blue calf (40 kg bodyweight) was born by cesarean section after a full term gestation. It died shortly after birth without any clear symptoms. The dam did well and recovered from the procedure without any complications. An abortion protocol which included necropsy, aerobic and fungal culture of the liver, culture for *Brucella abortus* spp. of the liver, PCR test for *Anaplasma phagocytophilum* and *Coxiella burnetti* of the spleen, ELISA test for *Bovine viral diarrhea virus* on skin ear biopsy and Stamp stain on liver was carried out. At necropsy, the thyroid gland was increased in volume (36.2 g) and the liver had an orange appearance. The lungs were inflated and the abomasum contained milk. Inspection of the abdomen revealed a congested, hemorrhagic, firm perirenal mass of 25x15 cm, which surrounded the entire left kidney (Figure 1). The kidneys appeared normal. The left kidney was attached to, but grossly not involved in the neoplastic process. No metastases were found in surrounding tissues or regional lymph nodes, more specifically the renal, sublumbar and iliacal lymph nodes.

No relevant pathogenic bacteria could be cultured on regular culture media. A culture for *Brucella spp.* and fungal organisms was negative, as was the Stamp staining (modified Ziehl- Neelsen stain) for acid fast bacteria (*Brucella spp, Chlamydia sp.* and *Coxiella burnetti*). The PCR test for Q-fever (*Coxiella burnetti*) and *Anaplasma phagocytophilum* was negative. The ELISA test for *Bovine pestivirus* antigens (*Bovine Viral Diarrhea Virus*) also showed to be negative. Analysis of a serum sample from the dam was negative for antibodies to *Brucella spp., Coxiella burnetti* (Q fever), *Leptospira hardjo* and *Neospora caninum*.

Histopathology and immunohistochemistry

For histopathology, specimens of the perirenal mass, liver and thyroid gland were fixed in 10% neutral buffered formalin and embedded in paraffin wax. Tissue samples were processed routinely. Four-µm-thick sections were mounted and stained with hematoxylin and eosin for histologic examination.

Histology showed a densely cellular, well-demarcated, partially encapsulated, expansile and non-infiltrative mass composed of round to polygonal cells organized in bundles and separated by bands of fibrovascular stroma (Figure 2). The cells had indistinct cell borders, a round to polygonal, basophilic nucleus with coarse chromatin and clear eosinophilic cytoplasm. There was moderate anisokaryosis and anisocytosis. Mitoses were 0-2 per high powerfield. Severe multifocal to diffuse hemorrhages were present throughout the mass. There was multifocal karyorrhexis and karyolysis of neoplastic cells(lytic necrosis) and marked infiltration of intact and degenerated neutrophils. Immunohistochemistry (IHC) was done to determine the cell origin.

Immunohistochemical staining was performed on formalin-fixed, paraffin embedded sections. Neoplastic cells were positive for vimentin (monoclonal mouse anti-Vimentin clone Vim 3B4, M7020, Agilent, Santa Clara, United States) (Figure 3), but negative for cytokeratin (monoclonal mouse anti-human Cytokeratin clone AE1/AE3, M3515, Agilent, Santa Clara, Unites States), desmin (monoclonal mouse anti-human desmin clone D33, M0760, Agilent, Santa Clara, United States), neurofilament (monoclonal mouse anti-human neurofilament protein clone 2F11, M0762, Agilent, Santa Clara, united States), CD3 (polyclonal rabbit anti-human T-cell, A0452, Agilent, Santa Clara, United States), CD20 (polyclonal rabbit anti-CD20, RB-9013-P, Thermo Scientific, Waltham,

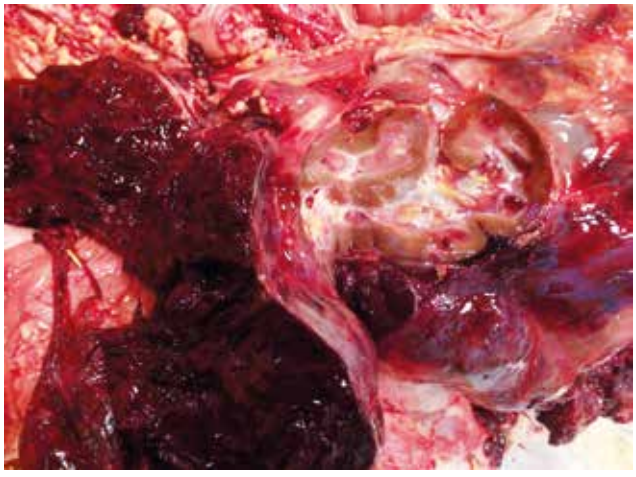

Figure 1. Hemorrhagic, firm perirenal mass of 25x15 cm surrounding the entire left kidney.

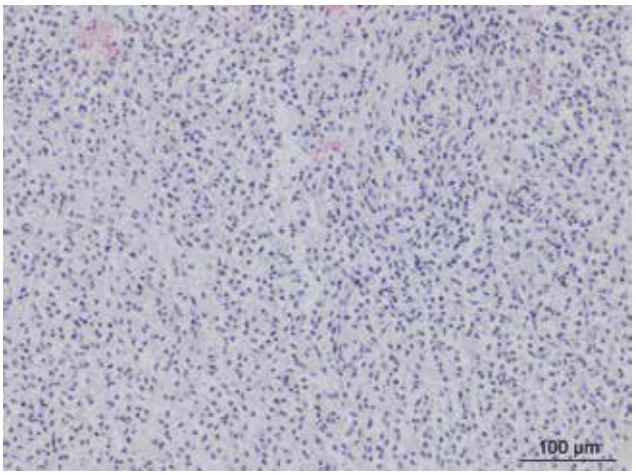

Figure 2. Neoplastic cells with indistinct cell borders, a round to polygonal, basophilic nucleus with coarse chromatin and clear eosinophilic cytoplasm. Note moderate anisokaryosis and anisocytosis.

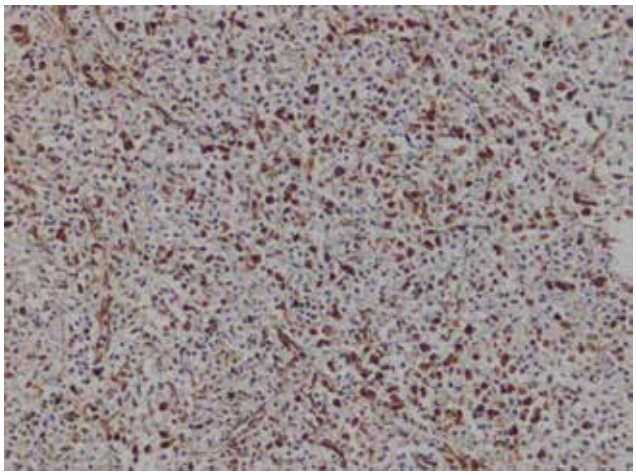

Figure 3. Most of the neoplastic cells show immunopositivity for vimentin immunohistochemical staining.

United States) and Von Willebrand Factor (polyclonal rabbit anti-human Von Willebrand Factor (A0082, Agilent, Santa Clara, United States). Mesotheliomas may have an epitheloid or sarcomatous appearance and mesothelial cells may express both cytokeratin and vimentin. Immunostainings for calretinin, keratin-5 and Wilms' tumor protein used in humans for identifying mesotheliomas are not suitable for diagnostic purposes in animals (Sato et al., 2005; Geninet et al., 2003; Bacci et al., 2006). According to the histologic pattern and the immunohistochemical findings, the tumor was identified as a fibrosarcoma. On histology, the kidney was not attached to or infiltrated by the mass. No significant changes were found.

The liver showed mild at random necrosis. Hepatocytes in this area were brightly eosinophilic and shrunken with marked karyorrhexis and -lysis (lytic necrosis). Necrotic areas were moderately infiltrated by degenerate neutrophils. There was marked accumulation of yellow to green pigment within the bile ducts and bile canaliculi (bile stasis). Within the portal areas, there was mild infiltration of mononuclear cells and mild proliferation of fibroblasts with production of collagen (fibrosis).

The thyroid gland showed marked congestion of the parenchyma with normal colloid producing follicles, lined by normal cuboidal to columnar follicular cells and separated by a network of interfollicular stroma, capillaries and normal parafollicular cells.

DISCUSSION

In case of a perirenal mass in bovine species, the differential diagnosis is wide. The following types of tumors have to be considered: congenital infiltrative lipoma or retroperitoneal lipoma, fibrochondrolipoma, malignant lymphoma, mesothelioma, hemangiosarcoma and nephroblastoma. Perirenal lipomas, nephroblastomas and mixed tumors often become quite large and can cause obstruction of the ureter. This results in hydronephrosis or causes dystocia due to ascites. Abdominal mesotheliomas usually cause large amounts of abdominal fluid, and therefore dystocia (Agerholm et al., 2016; Misdorp, 2002a). Malignant lymphoma and nephroblastoma have been reported quite frequently in both calves and children. In contrast to children, mesotheliomas are regularly reported in calves. Because neonatal tumors in children and animals such as calves have a similar pathological spectrum, a developmental origin has been suggested (Misdorp, 2002ab; Moore et al., 2013). Furthermore, since the fetal period is short compared to the total life span of the animal, it is expected that genetic factors rather than environmental factors play a role in the development of such tumors (Misdorp, 2002b; Moore, 2013; Sickinger et al., 2009). Nevertheless, environmental factors can influence the fetus. A study by Ortega-Garcia et al. (2012) in humans showed a causative relation between prenatal exposure to petrolium derivates and the occurrence of congenital fibrosarcomas in infants.

Given the relatively small size of the tumor and the absence of abdominal distention, it is unlikely that dystocia was the cause of death in the present case. The mass was hemorrhagic but no hemoabdomen was present, which also makes exsanguination an unlikely cause of death. Nevertheless, hemorrhagic diathesis has been reported in congenital infantile fibrosarcoma with anemia and thrombocytopenia, even in absence of overt bleeding (Mayssaa et al., 2013). More likely, the calf in the present case succumbed as a result of the random lytic necrosis that was present in the liver. This pattern is typical of many infectious agents including viruses and bacteria (Cullen and Brown, 2012). Bovine herpesvirus 1, an abortigenic herpes

virus, which can be transmitted by transplacental route, may have caused the random necrosis in the present case (Crook et al., 2012). However, following an eradication programme recognized by Europe, the herd was free from IBR since 2008 and had not experienced any clinical episodes of the disease. Unfortunately, fetal material was no longer available to test for IBR. Although bacterial examination of the liver was negative, bacterial septicemia or toxinemia (possibly intrauterine) and damage by bacteria originating from the gut should be taken into account as differential diagnosis for this type of necrosis. Toxic agents more likely cause midzonal or periportal necrosis, or in case of copper intoxication, the necrosis is rather centrilobular (Cullen and Brown, 2012). Intoxication seems less likely considering the pattern of necrosis and the age of the animal.

The increased volume of the thyroid gland in the present case was indicative for a congenital goiter. In lambs and cattle, congenital goiter is caused by iodine deficiency in utero (Leipold et al., 1990) and is commonly associated with abortion, stillbirth and birth of weak offspring (Capen, 1995; Seimya et al., 1991). In this case, a colloidal or hyperplastic goiter could not be confirmed on histopathological examination.

Although rare, congenital fibrosarcoma should be considered as a differential when confronted with hemorrhagic masses in aborted fetuses and newborn calves. In this study, the importance of immunohistochemical staining for diagnosis was demonstrated.

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Development of a non-functional pancreatic neuroendocrine tumor and a duodenal ulceration after cholecystoduodenostomy in a cat

Ontwikkeling van een niet-functionele neuro-endocriene pancreastumor en een duodenale ulcus na cholecystoduodenostomie bij een kat

[1]E. Bianchini, [2]N. Devriendt, [3]H. De Cock, [2]F. Mortier, [1]T. Rick, [2]D. Paepe, [2]H. de Rooster

[1] Department of Veterinary Medical Imaging and Small Animal Orthopedics, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium
[2] Small Animal Department, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium
[3] Medvet - Veterinary Pathology Services, Emiel Vloorsstraat 9, 2020 Antwerpen, Belgium

erika.bianchini@ugent.be

ABSTRACT

A six-year-old Ragdoll with previous extrahepatic biliary tract obstruction due to cholangiohepatitis, treated with cholecystoduodenostomy, was presented for acute vomiting, hyporexia, and weight loss. Abdominal ultrasound examination revealed randomly distributed hepatic nodules and dilated biliary ducts. Gastroduodenoscopy showed a patent cholecystoduodenostoma but disclosed a perforated duodenal ulceration. Conversion to celiotomy revealed extensive liver pathology, a discrete pancreatic nodule, and a duodenal ulcer opposite to the cholecystoduodenostoma. The cat was euthanized intra-operatively and necropsy was performed. The intrahepatic biliary tract of the right liver lobes was obstructed and severely dilated, whereas bile from the left lobes drained through the cholecystoduodenostoma. Histopathologic diagnoses were a primary pancreatic tumor, positive for glucagon on immunohistochemistry, with liver metastases, chronic purulent cholecystitis, and duodenal ulceration. To the authors' knowledge, this is the first report in which the development of pancreatic neoplasia is described in a cat with a history of biliary tract disease.

SAMENVATTING

Een zes jaar oude ragdoll met een voorgeschiedenis van cholecystoduodenostomie na extrahepatische galgangobstructie door chronische cholangiohepatitis werd aangeboden omwille van acuut braken, verminderde eetlust en gewichtsverlies. Op het abdominale echografisch onderzoek werden diffuus verspreide levernodules gezien en gedilateerde galgangen. Via gastroduodenoscopie werd een patente cholecystoduodenostomie opening gezien maar eveneens een geperforeerde duodenale ulcus. Tijdens de daaropvolgende exploratieve celiotomie werden uitgesproken afwijkingen ter hoogte van de lever vastgesteld en het darmulcer in de wand van het duodenum tegenover de cholecystoduodenostomieopening werd bevestigd. Daarenboven werd een kleine nodule ter hoogte van de pancreas opgemerkt. In overleg met de eigenaar werd de kat intraoperatief geëuthanaseerd waarna een necropsie werd uitgevoerd. De afvoer van de sterk gedilateerde intrahepatische galgangen van de rechterleverlobben was geblokkeerd. De gal afkomstig van de linkerleverlobben draineerde in het duodenum via de cholecystoduodenostomie-opening. De histologische diagnose was een primaire pancreastumor, die aankleurde voor glucagon op immunohistochemie, met levermetastases. Er was ook sprake van chronisch purulente cholecystitis en een duodenale ulcus. Volgens de auteurs is dit de eerste casuïstiek waarin de ontwikkeling van een neoplastisch proces wordt beschreven ter hoogte van de pancreas bij een kat met een voorgeschiedenis van galgangproblemen.

INTRODUCTION

Cholecystoduodenostomy is widely considered to be the best surgical procedure for biliary diversion in dogs and cats in case of obstruction or trauma affecting the extrahepatic biliary tree, when the gallbladder is not affected by the disease process (Doran and Moore, 2007; Morrison et al., 2008). Reports on long-term complications in cats are scarce and comprise stenosis of the stoma, reflux cholangiohepatitis, recurrent cholelithiasis, chronic weight loss, and duodenal ulceration, the last being observed after cholecystojejunostomy (Eich and Ludwig, 2002; Mayhew et al., 2002; Bacon and White, 2003; Buote et al., 2006; Doran and Moore, 2007). In cats, the close anatomical relationship between the pancreatic and biliary duct predisposes to concurrent pathologies (Mayhew et al., 2002; Jergens, 2012); however, it is often unclear which problem is causal, a consequence, or even coincidental. In human medicine, bile acids are suspected to play a role in the carcinogenesis of pancreatic tumors (Feng and Chen, 2016), but a similar association has not yet been described in cats. Moreover, the reported incidence of feline primary pancretic neoplasia is extremely low and pancreatic neuroendocrine carcinomas are even more rare (Seaman, 2004; Linderman et al., 2013). In the present case, the development of a pancreatic neoplasia and a duodenal ulceration are reported after previous cholecystoduodenostomy. To the authors' knowledge, this has not been reported previously in the veterinary literature.

CASE DESCRIPTION

A six-year-old, female Ragdoll was presented for acute vomiting, hyporexia and weight loss. Nine months earlier, cholecystoduodenostomy had been performed because of common bile duct obstruction; chronic suppurative cholangiohepatitis with dilation and proliferation of bile ducts and mild ulcerative, neutrophilic and plasma-cellular enteritis had been diagnosed and treated with long-term antimicrobial therapy. Although after recovery, the cat had been free of clinical signs till a few days before being presented again, she had experienced significant weight loss over time.

At presentation, a palpable mass in the cranial abdomen was detected. General blood analysis, including coagulation profile, did not show significant abnormalities. Abdominal ultrasound (US) revealed multiple ovoid, hyperechoic, well-defined nodules of various sizes (1-2 cm), randomly distributed over the entire hepatic parenchyma. The intrahepatic biliary ducts were moderately to severely dilated and contained partially echoic bile and focal hyperechoic areas (gas). The gallbladder was positioned over the duodenum and demonstrated a moderate amount of echoic bile in its lumen. The exact attachment and opening towards the duodenum were not clearly visualized due to gas content of the duodenum in this region; however, the opening appeared to be permeable for a few millimeters. No significant ultrasonographic abnormalities were observed in the remaining abdominal organs. Ultrasound-guided fine-needle aspirates of the liver nodules revealed several clusters of epithelial cells with augmented nucleus/cytoplasm ratio, mild to moderate anisocytosis and anisokaryosis. The bile, aspirated under US guidance, was non-pigmented and viscous, and contained fibrin-like debris. These characteristics belong to the definition of "white bile", a finding associated with biliary tract obstruction (Hashmonai et al., 1984). Microscopically, a large number of moderately degenerated neutrophils with numerous intra- and extracellular rod-shaped bacteria was present. Microbiological testing of the collected bile revealed the presence of *Escherichia coli*, sensitive to potentiated amoxicillin.

The main differential diagnosis was recurrent cholangiohepatitis and biliary tract (sub)obstruction with either reactive changes of the intrahepatic biliary tract epithelium (due to chronic bacterial infection) or with concurrent epithelial neoplasia. Treatment was initiated with amoxicillin-clavulanic acid (Kesium®, Ceva, Bruxelles, Belgium), ursodeoxycholic acid (Ursochol, Zambon, Bruxelles, Belgium), metoclopramide (Emeprid®, Ceva, Libourne, France), mirtazapine (Mirtazapine, Mylan, Bruxelles, Belgium) and tramadol (Tralieve, Dechra Veterinary Products NV, Lille, Belgium). Liver biopsies were advised to investigate possible neoplasia.

At the control visit twenty days later, hyporexia, occasional vomiting and weight loss were still present. On US, the bile was increased in echogenicity compared to the previous examination, and focal spots of gas were present in the region of the cholecystoduodenostoma. The gallbladder showed a moderate amount of echoic bile in its gravity dependent portion of the lumen. The stoma was difficult to visualize but appeared permeable. Because of the presence of white bile at the previous examination and the inability to see the stoma of the cholangioduodenostomy well with US, it was decided to perform gastroduodenoscopy. The endoscopic examination confirmed patency of the cholecystoduodenostoma but revealed ectasia at the duodenal wall opposite to the cholecystoduodenostomy site. In the center of this dilation, smooth white-colored tissue was surrounded by hyperemic edges, indicative for a duodenal ulcer. Because of the development of pneumoperitoneum with a sudden decrease in respiratory rate and saturation, the procedure was converted to ventral midline celiotomy. The right liver lobes were completely replaced by a multinodular mass composed of 0.5 cm up till 2 cm slightly umbilicated nodules, grey to yellow and firm with a necrotic center on the cut surface. The left liver lobes presented similar nodules, in less number. The omentum was adherent to the liver surface at several sites, to the gallbladder, and to the proximal duodenum. A discrete, irregular and hard nodule of 1 cm

was palpable in the right lobe of the pancreas. Due to the extensive liver pathology, the cat was euthanized intra-operatively.

At necropsy, once the omentum was released, a small perforation was seen in the mesenteric side of the duodenal wall, opposite to the cholecystoduodenostoma at the level of the duodenal ulcer (Figure 1). The cholecystoduodenostoma drained normal greenish bile, originating from the left liver lobes. The right bile ducts were obstructed and contained viscous white material. Samples of left and right liver lobes, duodenum and pancreas were taken for histopathological examination. The most important histologic findings were present in the liver lobes and pancreas. Within the pancreas, there was a poorly circumscribed infiltrative neoplastic mass composed of noduli of densely packed nests with moderately pleomorphic oval cells with oval nucleus with coarsely granulated chromatin and a moderate amount of well-defined eosinophilic cytoplasm. The neoplastic nests were surrounded by a small amount of collagen stroma. Mitoses were numerous, MC:34 (2.37 mm²).

Histologic examination of the right liver lobes revealed a diffuse infiltrative, widely disseminated neoplastic process composed of moderately circumscribed nodules with densely packed irregular tubular structures lined by a moderately pleiomorphic cuboidal epithelium, with a round to oval nucleus with coarsely granulated chromatin and a small amount of well-defined eosinophilic cytoplasm. The neoplastic tubules were surrounded by a small amount of collagen stroma. Mitoses were moderate; MC: 14 (2.37 mm²). In the remaining liver parenchyma, the bile ducts were surrounded by a concentric band of slightly mucinous collagen stroma with a mild to moderate infiltrate of neutrophils, macrophages and lymphocytes. Similar neoplastic nodules were present randomly distributed in the left liver lobes.

The neoplastic cells in the pancreas and liver resulted positive for chromogranin A (Chrom A 1/1000, Abcam, Cambridge, UK), pancytokeratin (CK Pan CK AE1/AE3, Dako, Glostrup, Denmark) and glucagon (Glucagon ab10988 Clone K79bB10, Abcam, Cambridge, UK), and negative for cytokeratin 7 (Cytokeratin 7, Dako, Glostrup, Denmark), insulin (Insulin RTU antibody, code IK002, Dako, Glostrup, Denmark) and gastrin (Gastrin GA519, Dako, Glostrup, Denmark). Based on these immunohistochemical characteristics in combination with the distribution of neoplastic nodules, the diagnosis of primary pancreatic neuroendocrine carcinoma, immunoreactive for glucagon, was made (Figure 2).

DISCUSSION

In this case report, a case of pancreatic neuroendocrine carcinoma with massive liver metastases and duodenal ulceration is described in a cat that had undergone a cholecystoduodenostomy nine months earlier.

The reported incidence of feline primary pancreatic neoplasia is extremely low and reports on pancreatic neuroendocrine neoplasia (pan-NEN), other than insulinomas, are even more rare, although the true incidence of pan-NEN might be higher than assumed (Seaman, 2004; Linderman et al., 2013). In general, pan-NENs are divided into two groups: functional (hormone-producing and -secreting) and non-functional neoplasms (Uribe Galeano et al., 2017).

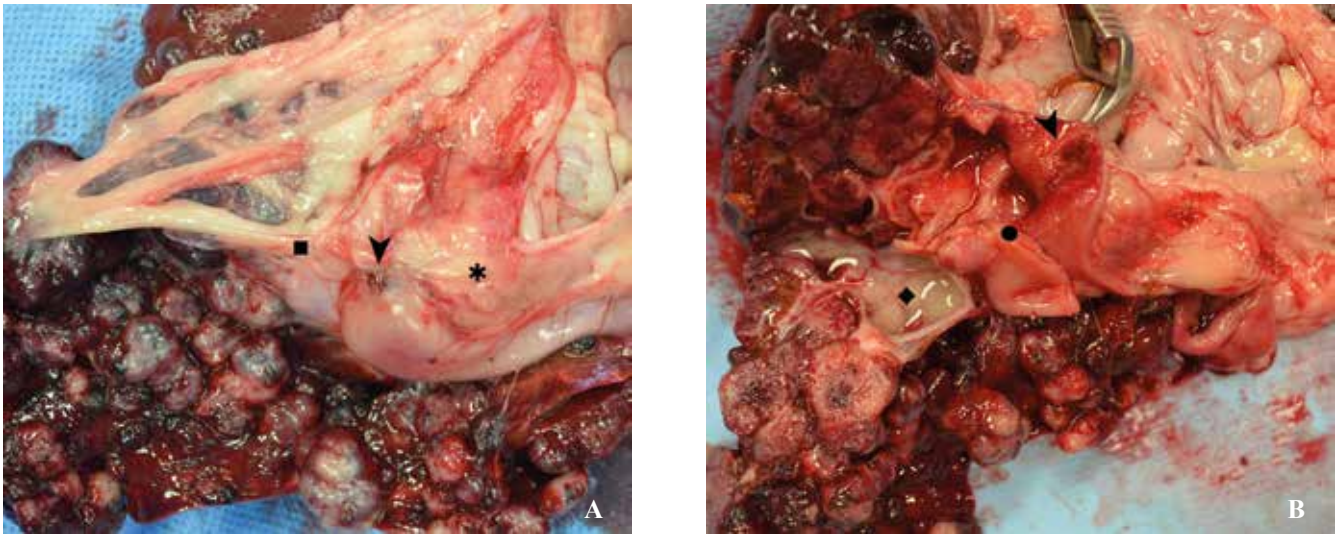

Figure 1. Post-mortem images of the cat ten months after cholecystoduodenostomy. A. The omentum was fixed to a liver metastasis and to the site of the duodenal ulcer (➤), opposite to the site where the gall bladder (■) was attached to the duodenal wall. A discrete nodule on the pancreas (✱) betrayed the underlying primary pancreatic neoplasia. B. After incising the liver parenchyma and the duodenum, patency of the cholecystoduodenostomy site (●) was confirmed. At the opposite site, the duodenal wall was ulcerated (➤).The severely dilated intrahepatic biliary tract of the right liver lobes contained white, viscous bile (♦).

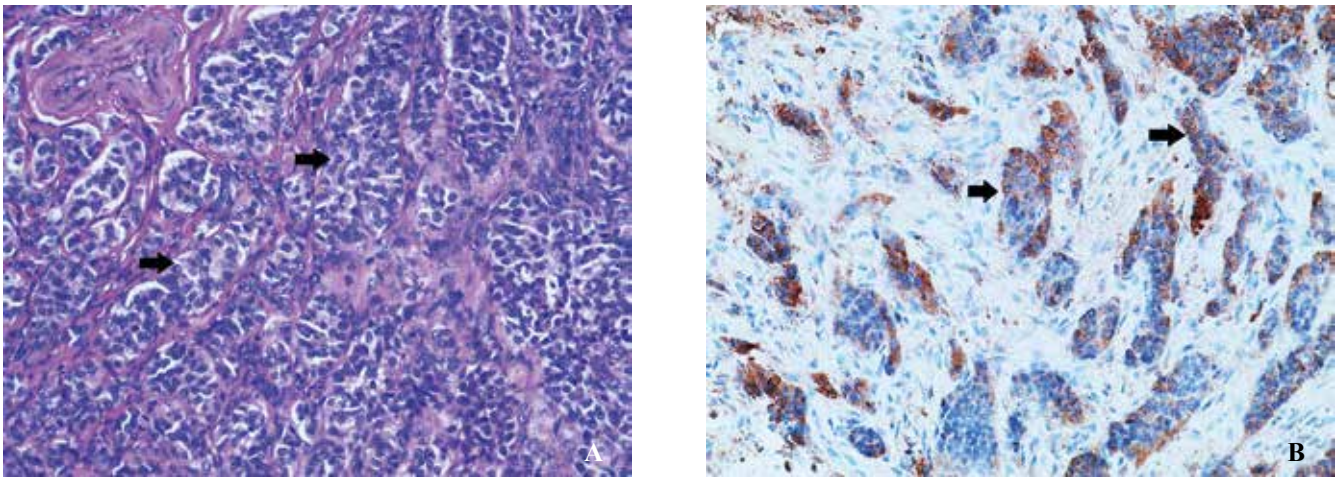

Figure 2. Histologic images of the pancreatic neuroendocrine neoplasia. A. Neuroendocrine tumor nests in the pancreas (arrow); H&E, (400x). B. Immunohistochemical glucagon-positive staining of the nests in the pancreas (arrow); IHC staining for glucagon, (200x).

As in humans (Halfdanarson et al., 2008), most are non-functional, eventually producing hormones, but without inducing clinically evident hormone-related syndromes (Seaman, 2004; Linderman et al., 2013). As a consequence, pan-NENs are generally incidental findings, unless they cause clinical signs due to a mass effect (Cloyd, 2015).

In the cat reported here, the pan-NEN stained negative for insulin and gastrin on immunohistochemistry but positive for glucagon. Glucagon-producing tumors are rarely reported in dogs and cats (Rosol and Meuten, 2017). In cats, one pan-NEN with exocrine differentiation (Michishita et al., 2017), one glucagonoma in a cat with necrolytic migratory erythema (Sahinduran and Ozmen, 2017), one glucagon-positive gastrin-secreting pancreatic tumor (Middleton and Watson, 1983), and one glucagon-producing hepatic neuroendocrine carcinoma (Asakawa et al., 2013) have been reported. In the present case, primary pancreatic neoplasia was not suspected clinically, related to the small and most likely non-functional pancreatic nodule, nor was it diagnosed by US, presumably because the small lesion was masked by the massive liver pathology and the previous cholecystoduodenostomy. Furthermore, the difference between acute pancreatitis and pancreatic neoplasia is difficult to detect by US (Bennett et al., 2001). In the past, any tumor staining positive for glucagon on immunohistochemistry was called glucagonoma, but, according to recent WHO guidelines on tumors of the digestive system, tumors in the absence of typical clinical syndromes should nowadays be termed non-functional NENs (Nagtegaal et al., 2020).

Only during necropsy, it became clear that the white bile obtained via US-guidance was not collected from the gallbladder itself (as this drained normal green bile coming from the left liver lobes), but from a part of the dilated right extrahepatic biliary tracts. This error prompted to proceed with further investigation, which otherwise would possibly have been postponed.

Liver metastases are frequent findings in human pancreatic glucagonomas (Soga and Yakuwa, 1998), either with or without diabetico-dermatogenic syndrome (DDS and metastases were also present in two of the reported feline pancreatic endocrine tumors) (Middleton and Watson, 1983; Michishita et al., 2017). In human medicine, metastatic rate seems to correlate with the size of the primary tumor and its malignancy (Soga and Yakuwa, 1998). In the cat of this present case, the liver masses stained positive for glucagon during immunohistochemistry, confirming the diagnosis of a primary pancreatic neoplasia. However, macroscopically, the dimension and severity of the liver lesions compared to the pancreatic nodule rather suggested a primary liver neoplasia.

Based on the absence of typical clinical symptoms, it can be assumed that this feline pan-NEN was non-functional. A test for blood glucagon is not readily available for cats. Serum biochemistry did not reveal indirect signs of hyperglucagonemia (e.g. hyperglycemia, anemia, hypoaminoacidemia). Necrolytic migratory erythema, a syndrome associated in humans (Tierney and Badger, 2004), dogs (Gross et al., 1993), and two cats with glucagonoma (Asakawa et al., 2013; Sahinduran and Ozmen, 2017) was not observed.

It might not have been a coincidence that the pancreatic tumor in this cat was observed after biliary tract disease. The strong anatomical connection between pancreas and gall bladder, especially in the feline species, predisposes to mutual pathologic influences, but a clear physiological basis has not yet been established (Jergens, 2012). Since the feline extrahepatic biliary anatomy closely resembles the human situation (Mayhew et al., 2002), abnormal bile acid flow combined with severe bacterial cholangiohepatitis could have promoted neoplastic changes in the pancreas similar to what has been described in hu-

mans (Cigrovski Berkovic et al., 2014). It has been suggested that bile acids systemically activate cancer-signalling pathways, but also locally induce pancreatic carcinogenesis, although typically, pancreatic adenocarcinoma would be expected rather than neuroendocrine neoplasia (Feng and Chen, 2016). At the time of the previous surgery, the pancreas had appeared normal and liver biopsies had tested negative for malignant disease, but had revealed chronic suppurative cholangiohepatitis, which could also have triggered carcinogenesis. In humans, chronic inflammation and pro-inflammatory cytokines have been shown to be modifying factors in tumor growth (Cigrovski Berkovic et al., 2014).

An additional finding in the cat of the present case was the ulcer opposite the cholecystoduodenostomy site. Duodenal ulcer formation is a known complication after cholecystojejunostomy (Doran and Moore, 2007; Mehler, 2011). When bile is diverted from the duodenum to the jejunum via a rerouting procedure, gastric acid secretion by the stomach is increased while there is no longer bile for neutralization of gastric acids in the duodenum, leading to ulcerative damage to the proximal duodenum (Davies et al., 1985). Cholecystoduodenostomy theoretically does preserve the neuroendocrine reflex of acid inhibition and is therefore preferred (Mehler, 2011).

The question why a duodenal ulcer was formed in this case and, in particular, whether its localization was incidental, is difficult to answer. Firstly, it can be hypothesized that the amount of bile reaching the duodenum was insufficient to neutralize the gastric acids (Mehler, 2011), since bile of the right liver lobes was no longer drained. It could be argued whether the constant "dripping" of bile onto the contralateral duodenal wall, due to the absence of a sphincter regulating the bile flow, may have resulted in a mechanical damage with disruption of the normal architecture. Lastly, duodenal ulcers have been related to a specific type of pan-NEN, gastrinoma, and its associated Zollinger-Ellison syndrome; this type of tumor has previously been reported in a cat with duodenal ulcers (Middleton and Watson, 1983). In the present case, immunohistochemistry of the tumor tested negative for gastrin. Nevertheless, it is known that pan-NENs can stain positive for several pancreatic hormones, with predominance of one hormone. Furthermore, correlation between immunohistochemistry and bioactivity is not always apparent (Rosol and Meuten, 2017). Therefore, a role of gastrin in the pathogenesis of the duodenal ulcer cannot completely be excluded in the present case.

In conclusion, abnormal bile acid flow combined with severe bacterial cholangiohepatitis could have promoted the development of the pancreatic neoplasia, similar to bile acid involvement suggested in the carcinogenesis of human pancreatic cancer. Formation of the duodenal ulcer opposite to the cholecystoduodenostoma might have been caused by insufficient neuroendocrine acid inhibition and/or by irritation due to constant contact with bile. Based on both findings, pancreatic neoplasia and duodenal ulceration opposite to the cholecystoduodenostoma should be considered in the differential diagnosis of cats that clinically deteriorate after prior recovery of biliary tract surgery.

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Uit het verleden

Paard eten

Vanouds, met name in de middeleeuwen, werd het paard geassocieerd met de adel – was zelfs aan de adel voorbehouden - en speelde het een belangrijke rol in de cavalerie. Door deze bijzondere positie was er ook vroeger al duidelijk weerstand tegen het eten van paardenvlees, behalve als het om oude afgeleefde werkpaarden ging. Paarden, zeker deze waarop ridders reden hadden eigennamen en waren wijd en zijd bekende persoonlijkheden. (…). Een paard heeft dan ook geen poten maar ‘benen’, geen kop, maar een ‘hoofd’. Kortom het staat tamelijk dicht bij de mens.

Uit: Lemaire T. (2017). *Onder dieren*, Ambo/Anthos, Amsterdam, p. 22

Luc Devriese

Putative paraneoplastic pemphigus in a dog: clinical and microscopic findings

Mogelijke paraneoplastische pemfigus bij een hond: klinische en microscopische bevindingen

[1]J. Declercq, [1]L. Declercq, [2]G. Vercauteren

[1]Small Animal Practice Jan Declercq, Poortersstraat 16-18, B-8510 Marke, Belgium
[2]Vet-Path bvba, Kruisken 9, B-9991 Adegem, Belgium

jan.declercq@dierenarts-jandeclercq.be

ABSTRACT

In this case report, a dog with clinical and histopathological features of paraneoplastic pemphigus is described. A Lhasa apso with severe ulcerative oral and predominant facial skin disease had a thoracic mass histopathologically diagnosed as a thymoma. A concurrent disease-association was suspected. Cytologic examination of the oral lesions provided early clues to the dog's ulcerative condition.

SAMENVATTING

In dit artikel wordt een hond beschreven met klinische en histopathologische symptomen van paraneoplastische pemfigus. Een lhasa apso met ulceratieve stomatitis en een voornamelijk faciale dermatitis vertoonde een thoracale massa, histopathologisch gediagnosticeerd als een thymoom. Een oorzakelijke associatie van de huidaandoening en het thymoom werd verondersteld. Het cytologisch onderzoek van de orale letsels was een vroege aanwijzing voor een mogelijke diagnose van paraneoplastische pemfigus.

INTRODUCTION

Paraneoplastic pemphigus (PNP) is a very rare, severe ulcerative autoimmune disease affecting the mucosa and mucocutaneous junctions. It is seen in conjunction with neoplasia and is considered a 'marker' of internal disease (Olivry, 2004; Gross et al., 2005; Elmore et al., 2005). The histopathology of the skin lesions reveals suprabasilar epithelial acantholysis typical of pemphigus vulgaris (PV), as well as keratinocyte apoptosis with satellitosis resembling lesions of erythema multiforme (EM). Mild lymphocytic interface dermatitis has been described in the literature (Olivry, 2004).

Three cases of PNP have been reported in dogs, one with a thymoma (Stannard et al., 1975), one with a thymic lymphoma with hepatic metastasis (Lemmens et al., 1998) and one with a splenic sarcoma (Elmore et al., 2005). In a meta-analysis of three dogs with PNP, all three exhibited skin lesions for two to four weeks prior to diagnosis (Olivry, 2004). In all dogs, there were extensive erosions and ulcers in the oral cavity, at mucocutaneous junctions as well as in haired skin. The lesions originated in the oral cavity, and oral involvement was always severe. The dogs exhibited halitosis, hypersalivation and anorexia. Systemic signs consisted of hyperthermia, lethargy and depression.

Two cases have been described in dogs with typical clinical and histological features of PNP (Olivry et al., 2000; Gross et al., 2005). The dogs showed no evidence of neoplasia upon systemic evaluation. A variant of spontaneous PV was the proposed diagnosis in one of the dogs (Olivry et al., 2000), and drug reaction was suspected in the other dog (Gross et al., 2005).

In the present paper, the clinical and microscopic findings in a dog with putative paraneoplastic pemphigus are described.

CASE DESCRIPTION

An eight-year-old, intact, male Lhasa apso was referred for diagnosis and treatment of a severe and painful ulcerative condition of a two-months duration, involving the oral cavity, and perioral and nasal mucocutaneous junctions. Up till then, treatment had consisted of a variety of antibiotics perorally and a dental care procedure with poor results.

On admission, the dog was lethargic, drooling, anorectic and had a rectal temperature of 39.3°C. Physical examination revealed an oral and predominantly facial skin disease. Severe ulceration was present on the tongue ('sloughing' glossitis and formation of pseudomembranes), hard and soft palate, buccal and labial mucosa, haired and non-haired lips and chin (Figure 1). Less severe ulceration was observed at the mucocutaneous junctions of the nose, around the eyes and on the forelimbs. Tentative clinical diagnoses were erythema multiforme, drug reaction, autoimmune skin disease and epitheliotrophic T-cell lymphoma.

Initial laboratory tests included a blood urea and creatinine evaluation. Both values were in the normal reference range and the dog was anesthetized for further examination. Cytologic examination was performed on scraping smears of labial mucosa obtained by means of a curette. In all samples, there was a predominance of epithelial cells, which were accompanied by a purulent inflammation, moderate numbers of small lymphocytes and eosinophils, and a small number of mast cells. Epithelial cells were observed in large sheets or clusters and as numerous individual cells. Small lymphocytes were frequently arranged around rounded single epithelial cells (satellitosis) (Figure 2). Neutrophils with intracytoplasmatic cocci were also present. Biopsy specimens of labial mucosa and lips were obtained. While awaiting the results of the histopathological examination, the dog was treated with oral prednisolone (Prednisolone, Kela Laboratories, Sint-Niklaas, Belgium) at 1.5 mg/kg once daily and with cefovecin (Convenia, Zoetis Belgium SA, Louvain-la-Neuve, Belgium) 8 mg/kg one-time only subcutaneously. Histopathology of mucosal and skin biopsies revealed mixed morphologic patterns of suprabasilar acantholysis consistent with pemphigus vulgaris (PV) and scattered individual keratinocyte apoptosis and satellitosis consistent with erythema multiforme (EM). Suprabasilar acantholysis with residual basal cells and irregular acantholysis affecting multifocally the lower spinosum, resulted in large clefts that often had numerous acantholytic cells within the lumen of the cleft (Figures 3 and 4). Keratinocyte apoptosis was not always associated with satellitosis and was not often concurrently observed with epidermal clefting (Figure 5). Some sections had numerous eosinophils within the lesional epidermis and adjacent superficial dermis (Figure 6). Lymphocytic interface dermatitis was not identified. The dermis or submucosa contained an infiltrate of lympho-

Figure 1. Tongue of the Lhasa apso at initial presentation. Severe ulcerative glossitis and ulceration of the oral mucosa are present. Note the yellowish sheets of sloughed lingual mucosa (pseudomembrane formation).

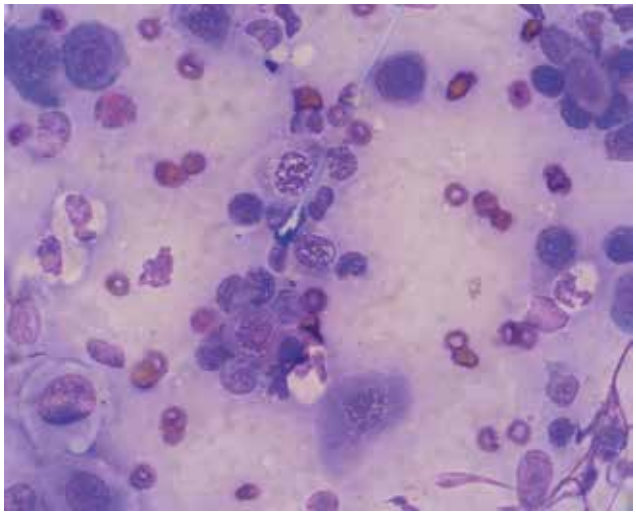

Figure 2. Cytologic view of a scraping smear of labial mucosa of the Lhasa apso. Note the presence of four rounded individual epithelial cells with lymphocytic satellitosis. Diff-Quik stain 400x.

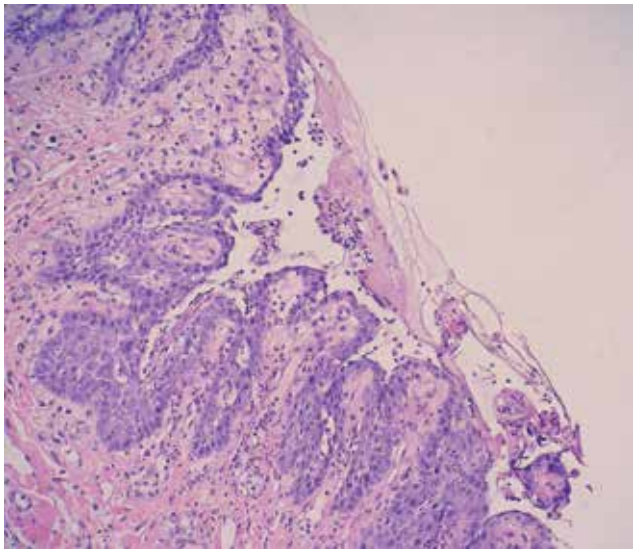

Figure 3. Photomicrograph of the lip of the Lhasa apso. Suprabasilar acantholysis and acantholysis affecting the lower spinosum.

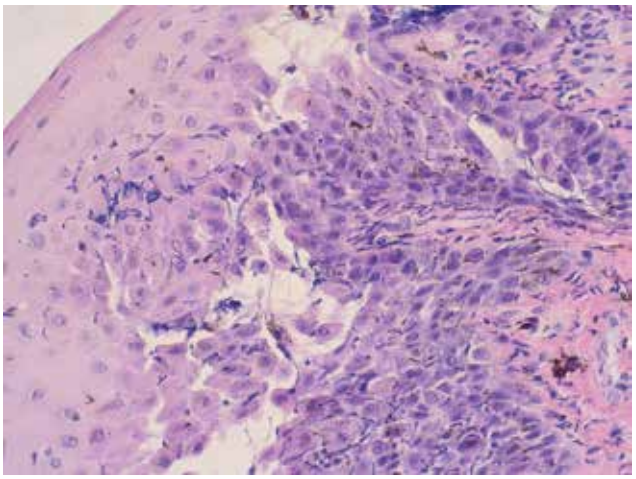

Figure 4. Photomicrograph of the oral mucosa of the Lhasa apso. Irregular acantholysis affects the lower spinosum, leaving multiple layers of keratinocytes on the floor of the cleft. Note the presence of numerous acantholytic cells within the lumen of the cleft. Hematoxylin and eosin stain 400x.

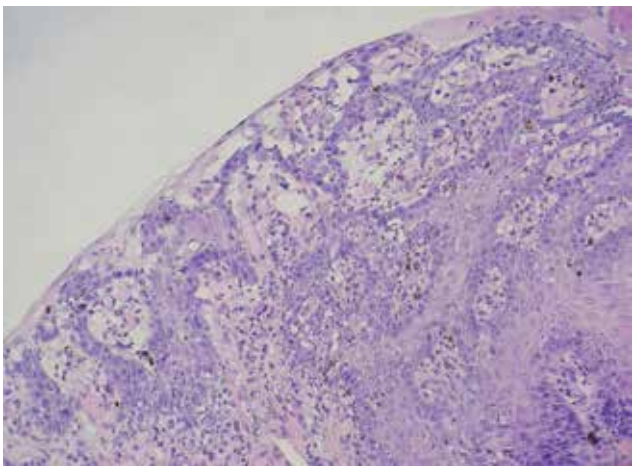

Figure 5. Photomicrograph of the lip of the Lhaso apso. Note suprabasilar acantholysis and scattered keratinocyte apoptosis with and without lymphocytic satellitosis. Note the presence of eosinophils within the dermal infiltrate. Hematoxylin and eosin stain 200x.

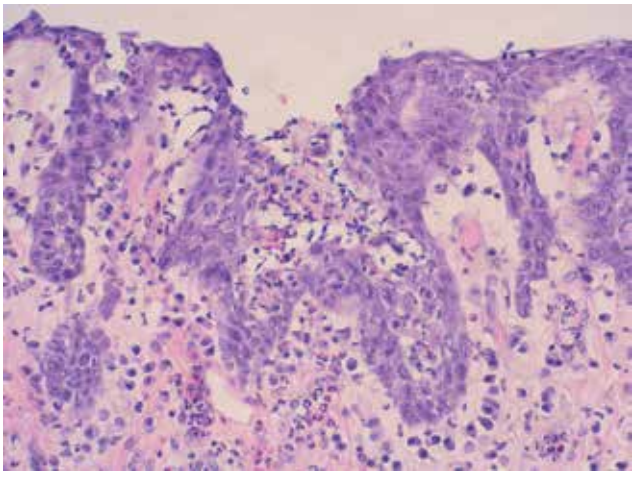

Figure 6. Photomicrograph of the lip of the Lhasa apso, illustrating the presence of numerous eosinophils in lesional epidermis and subjacent superficial dermis. Hematoxylin and eosin stain 400x.

cytes, macrophages and plasma cells. Occasionally, in some sections, eosinophils predominated the dermal infiltrate.

Clinical and microscopic findings were considered consistent with a diagnosis of PNP. The dog was screened for the presence of an internal neoplasia. Abdominal ultrasound did not reveal any abnormalities. Radiography of the thorax revealed a large mass in the cranial thorax (Figure 7). Ultrasound guided fine-needle aspiration of the mass was performed. Tissue samples were of low cellularity and revealed small, well-differentiated lymphocytes; no epithelial cells were found to support the presumptive diagnosis of thymoma.

At that time, the cutaneous signs of the skin condition had already been present for five months, and the dog did not show respiratory signs. The extent and severity of the lesions had continued to worsen despite treatment with glucocorticoids. The dog exhibited a severe ulcerative dermatitis affecting the oral mucosa, mucocutaneous junctions of the face, chin, ventral neck and forelimbs. The footpads were unaffected (Figures 8 and 9). The owner declined surgical removal of the thoracic mass and requested euthanasia. Post-mortem examination revealed a multilobulated and cystic mass in the cranial mediastinum. The histopathological diagnosis was thymoma.

DISCUSSION

In this paper, a dog presenting with clinical and histopathological features of PNP is described. The dog had a severe and painful ulcerative condition involving the oral cavity, mucocutaneous junctions as well as haired skin. Lesions included erythema and extensive ulceration. Severe oral ulceration is a hallmark of PNP in the dog. The histopathologic findings of suprabasilar acantholysis and keratinocyte apoptosis were consistent with the diagnosis of PNP, in which mixed morphologic patterns are typically found and mostly consist of concurrent microscopic features of both pemphigus vulgaris and erythema multiforme (Gross et al., 2005). Suprabasilar acantholysis was not often strictly above the basal cell layer in the dog of the present report, as is seen in classical pemphigus vulgaris, but also affected the lower spinosum, a microscopic feature of PNP, which has been recognized in the dog (Gross et al., 2005). In classical pemphigus vulgaris, suprabasilar clefts contain occasionally free-floating acantholytic keratinocytes (Gross et al., 2005). The histopathological findings in the dog of the present report were characterized by epithelial clefts with numerous free keratinocytes in the lumen. All these findings differed from ‘classic’ PV; hence, the term PV-like condition could be considered in this case. Lymphocyte-rich interface dermatitis with apoptosis of basal and suprabasilar keratinocytes is a hallmark of EM. In two cases of PNP, only mild lymphocytic interface dermatitis has been described (Lemmens et al., 1998; Elmore et al., 2005). Erythema multiforme

associated with thymoma and treated with thymectomy has also been reported in a dog with hyperemic stomatitis and skin lesions (Tepper et al., 2011). Histopathology of the erythema multiforme case revealed individual keratinocyte necrosis and hydropic degeneration of the basal layer of the epidermis and florid interface. These findings are consistent with 'classic' EM. As true lymphocytic interface dermatitis was not observed in the present case, lymphocytic cytotoxic dermatitis is a more appropriate term. A remarkable microscopic finding in cytologic and histologic samples of the dog of this report, was the presence of eosinophils. The dog had no history of allergic skin disease or parasitic dermatitis. The role of eosinophils in the pathogenesis of its skin condition is not clear.

Histopathology and immunologic studies, including the identification of targeting antigens, as well as the documentation of underlying neoplasia, are required for the diagnosis of PNP (Gross et al., 2005). Direct and indirect immunofluorescence studies were not performed in the present report as these techniques are not routinely available. To obtain a definitive diagnosis of PNP and to prove that the skin lesions and neoplasia are not two separate entities, the disappearance of the skin lesions with tumor removal and their recurrence with tumor regrowth should be demonstrated (Elmore et al., 2005; Hill et al. 2013). In the dog of this report, the requested euthanasia of the patient precluded this type of clinical assessment.

Cutaneous cytology is a valuable tool in veterinary dermatology and is performed routinely in acantholytic skin diseases, such as pemphigus foliaceus and pemphigus erythematosis. Cytologic examination of skin lesions in cases of pemphigus vulgaris is considered unrewarding due to the absence or low number of acantholytic epithelial cells. In the diagnostic approach of the dog in the present report however, cytologic examination of scraping smears of oral mucosa obtained by means of a curette, provided early clues to the dog's ulcerative condition. The presence of numerous non-neoplastic single and rounded epithelial cells in cytologic samples may suggest an acantholytic skin disease, and the presence of epithelial cells with lymphocytic satellitosis is supportive of concurrent cytotoxic dermatitis. Cytology of oral lesions has been performed in one case of PNP (Lemmens et al., 1998). Microscopic examination in this case did not reveal the presence of acantholytic keratinocytes. The collection technique is crucial in obtaining representative cells from the primary lesion. Scraping smears generally produce smears of greater diagnostic quality than impression smears (Cowell et al., 1989). Early recognition of PNP by cytologic examination of oral lesions can allow early tumor removal and immunosuppressive treatment.

In conclusion, dogs with PNP may present with PV-like and EM-like (cytotoxic) histopathological features. Routine performance of cytologic examination of oral lesions, using a proper technique of sampling, may provide early diagnostic clues to this very rare entity.

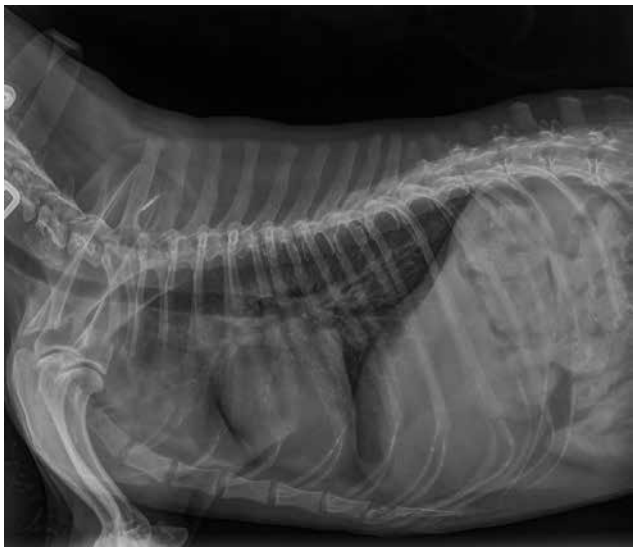

Figure 7. Left lateral radiographic view of the thorax of the Lasa apso, showing a large cranial mediastinal mass.

Figure 8. Lesion progression of the Lhasa apso. Note the mucocutaneous distribution of facial lesions and involvement of nasal mucosa.

Figure 9. Lesion progression in the Lhasa apso. Widespread ulceration is present on the chin, ventral neck and forelimbs.

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Surgical treatment of refractory incontinence in the bitch

Chirurgische behandeling van refractaire incontinentie bij de teef

J. Timmermans, B. Van Goethem, H. de Rooster

Small Animal Teaching Hospital, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

bart.vangoethem@ugent.be

ABSTRACT

Urinary incontinence is a common condition in spayed, female dogs with a reported prevalence between 3.1% and 20.1%. In the majority of dogs with acquired urinary incontinence, urethral sphincter mechanism incompetence is the underlying cause. Approximately 15% of bitches that initially respond to medical therapy ultimately become refractory. Surgical intervention is indicated when patients do not respond or become refractory to medical treatment. Based on the current literature, placement of an artificial urethral sphincter, i.e. an inflatable cuff around the proximal urethra connected to a subcutaneous injection port, provides a very reliable and long-term incontinence resolution in bitches and has a low complication rate.

SAMENVATTING

Urinaire incontinentie is een vaak voorkomende aandoening bij gesteriliseerde teven met een gerapporteerde prevalentie tussen 3,1% en 20,1%. Bij de meeste honden met verworven urinaire incontinentie is er sprake van een onderliggende urethrale sfinctermechanisme-incompetentie. Ongeveer 15% van de teven die initieel verbeteren met medicamenteuze behandeling, wordt uiteindelijk refractair. Chirurgische interventie is aangeraden wanneer patiënten niet verbeteren ondanks medicamenteuze behandeling of refractair worden aan medicamenteuze behandeling. Gebaseerd op de huidige literatuur biedt de plaatsing van een artificiële urethrale sfincter, dit is een opblaasbare "cuff" rondom de proximale urethra die verbonden is met een subcutane injectiepoort, een zeer betrouwbare en langdurige oplossing voor incontinentie bij teven, waarbij zelden complicaties optreden.

INTRODUCTION

In spayed, female dogs, urinary incontinence (UI) is a common condition with a reported prevalence between 3.1 and 20.1% (Arnold et al., 1989; O'Neill et al., 2017). The majority of dogs with acquired UI suffer from urethral sphincter mechanism incompetence (USMI) (Byron et al., 2017). The first treatment option in dogs affected by acquired UI is medical management. Although some cases become refractory to treatment, medical management is highly efficient in many cases. (Applegate et al., 2018). Urinary incontinence and subsequent medical management have been discussed in more detail in a previous literature review article published in this journal (Timmermans et al., 2019).

Historically, different surgical options have been researched. In this review, indications for surgical treatment in patients with acquired UI due to USMI are highlighted and the currently available surgical options are discussed.

SURGICAL TREATMENT

Surgical treatment is indicated 1. when patients do not respond to medical treatment or encounter severe adverse effects from it, 2. to treat refractory urinary incontinence, or 3. when owners are reluctant to administer lifelong medication. A large number of dogs with USMI are eventually presented as candidates for surgical treatment, since approximately 15-20% of

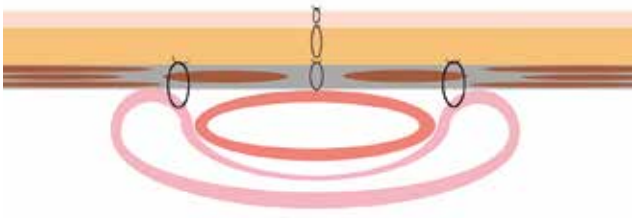

Figure 1. Schematic representation of the colposuspension technique illustrating how the vaginopexy (pink) compresses the urethra (orange) to the abdominal wall (grey).

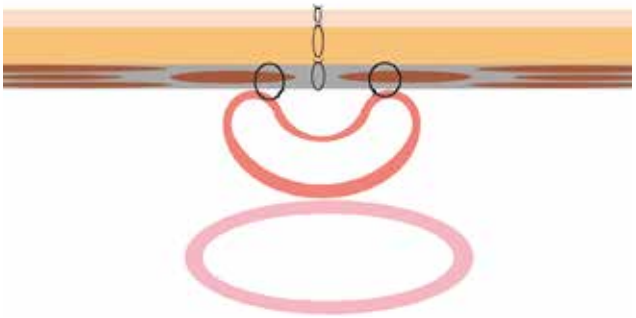

Figure 2. Schematic representation of the urethropexy technique illustrating how the procedure is reducing the diameter of the urethral lumen (orange).

bitches that initially respond to medical therapy ultimately become refractory (Scott et al., 2002; Adin, 2014). Additionally, until recently, most surgical procedures could not provide long-term continence; however, technological advances seem to have eliminated this problem.

Colposuspension

Colposuspension is essentially a vaginopexy that entraps the urethra between the vagina and the abdominal wall using nonabsorbable, monofilament suture material (Mcloughlin and Chew, 2009; Claeys et al., 2010b). Increased external compression of the proximal urethra is caused both by the compressive effect from the vaginopexy and also by the repositioning of both the bladder neck and proximal urethra into a more cranial intra-abdominal position (Rawlings et al., 2001; Applegate et al., 2018) (Figure 1).

Short-term continence is achieved in approximately 55% of cases. However, in a study by Rawlings et al. (2001), continence decreased to 14% one year postoperatively (n = 23 dogs). Additional medical therapy resulted in regained continence in a total of 38% of dogs after one year (Rawlings et al., 2001) (Table 1).

Major complications, including (partial) urethral obstruction, are rare (Rawlings et al., 2001; Mcloughlin and Chew, 2009). Transient dysuria is the most commonly observed minor complication.

Table 1. Data summary of surgical treatment options of refractory incontinence other than the artificial urethral sphincter.

	n	Follow-up (months)	Short-term continence		Long-term continence		Complications		Owner satisfaction
			SX	SX + MED	SX	SX + MED	Minor	Major	
Colposuspension									
Rawlings et al. (2001)	23	12	55%	N/A	14%	38%	N/A	N/A	83%
Urethro(cysto)pexy									
Massat et al. (1993)	10	14	N/A	40%	10%	30%	20%	0	60%
White (2001)	100	54	N/A	N/A	56%†	-	20%	3%	77%†
Urethropexy and colposuspension									
Martinoli et al. (2014)	30	39	N/A	N/A	70%	N/A	10%	0	97%
Urethral bulking									
Barth et al. (2005)	40	12	68%	83%	28%	65%	15%	0	70%
Bartges and Callens (2011)	22	1	77%	-	N/A	N/A	18%	0	N/A
Byron et al. (2011)	21*	56	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Transobturator vaginal tape									
Claeys et al. (2010)	7	11	71%	85%	71%	85%	14%	0	85%
Deschamps and Roux (2015)	12	48	92%	N/A	25%	-	58%	25%	83%
Hamon et al. (2019)	12	85	83%	-	40%‡	80%‡	17%	0	80%‡

Short-term continence < 6 months; long-term continence > 6 months, SX: surgery, SX + MED: surgery and medication, N/A: data not available in the publication, *Study on a total of 31 dogs of which 21 patients with USMI, †40 out of 100 dogs were excluded from long-term analysis, ‡2 out of 12 dogs were excluded from long-term analysis.

Urethro(cysto)pexy

Urethropexy consists of attaching the urethra to the ventral abdominal wall whereas with cystourethropexy, both the urethra and the bladder are attached to the abdominal wall (Mcloughlin and Chew, 2009). Nonabsorbable monofilament sutures are used in these techniques (Mcloughlin and Chew, 2009). During both procedures, the bladder neck and proximal urethra are repositioned more cranially in the abdomen. When performing a urethropexy, the diameter of the urethral lumen is reduced, creating higher urinary flow resistance (Massat et al., 1993; Mcloughlin and Chew, 2009) (Figure 2).

A prospective study on urethropexy performed by White (2001) revealed 87% short-term improvement (n = 100 dogs). However, long-term continence decreased to 56% approximately three years postoperatively (Table 1). Unfortunately, the effects of additional medical treatment following a urethropexy were not evaluated in the study by White (2001). Results after cystourethropexy are less positive than the urethropexy results, with only 10% continence after surgery and 30% long-term continence when phenylpropanolamine was administered (n = 10 dogs) (Massat et al., 1993).

Major complications are rare, with 3% of affected dogs requiring revision surgery due to anuria or avulsion of the urethropexy site (White, 2001; Martinoli et al., 2014). Minor complications are common, occurring in 20% of affected dogs, and include transient pollakiuria and dysuria.

Martinoli et al. (2014) described the combination of urethropexy and colposuspension in female dogs with refractory UI (n = 30 dogs) and found higher success rates compared to the rates found when either of these techniques were used alone. In that study, the combination therapy achieved long-term continence in 70% of dogs after a median follow-up of approximately 39 months. Complications were mild and temporary in 10% of cases. Remarkably, combining these two techniques is likely to decrease the risk of avulsion; this combination has been successful in bitches with previous avulsion of the soft tissues (Martinoli et al., 2014).

Urethral bulking

Urethral bulking is a minimally invasive treatment method where bulking agents are injected into the urethral submucosa (Arnold et al., 1996). Under videoendoscopic guidance, the bulking agents are injected in the proximal urethra at the 2-, 6-, and 10-o'clock positions to increase the urethral closure pressure (Klarskov and Lose, 2008; Byron et al., 2011; Applegate et al., 2018) (Figure 3). Bovine glutaraldehyde cross-linked collagen used to be the gold standard (Bartges and Callens, 2011; Byron et al., 2011), but is no longer available on the European market (Lüttmann et al., 2019). In a study by Bartges and Callens (2011), polydimethylsiloxane was used as an alternative. In that study, 22 female dogs, of which 77% were continent, were included; there was a follow-up period of only one month (Bartges and Callens, 2011). Recently, continence results after endoscopic urethral injections of dextranomer/hyaluronic acid copolymer have been reported by Lüttmann et al. (2019). Their findings identified the new copolymer as a valuable substitution. However, it provided a shorter duration of continence than bovine glutaraldehyde cross-linked collagen (Lüttmann et al., 2019).

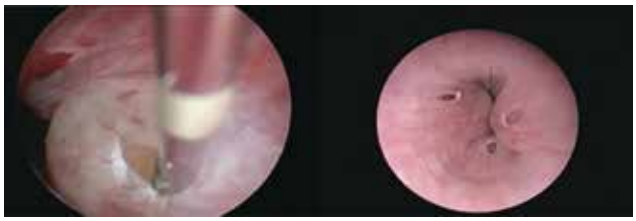

Figure 3. Videoendoscopic view of urethral bulking during (left) and after (right) injection of bulking agents (From: Applegate et al., 2018).

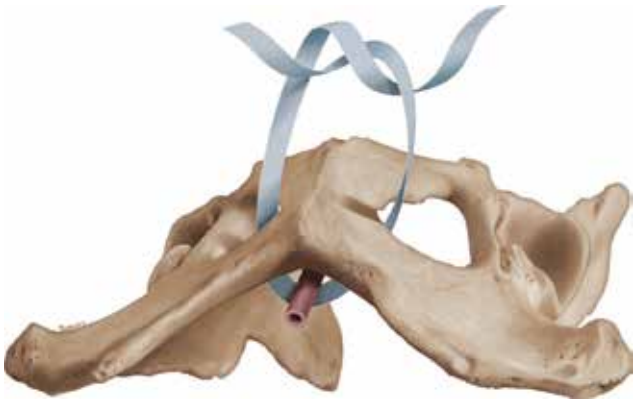

Figure 4. Schematic representation illustrating the placement of the transobturator vaginal tape using the inside-out variant (From: Deschamps and Roux, 2015).

Efficacy of solo-treatment with urethral injections is approximately 60-70%, but rises to 83% when dogs simultaneously receive alpha-adrenergic agonists, such as phenylpropanolamine (Barth et al., 2005; Byron et al., 2011) (Table 1). Over time, migration and flattening of the material in the mucosa occur, leading to a 40%-decrease in efficacy after one year of injecting collagen and the recurrence of UI (n = 40 dogs) (Barth et al., 2005). To assure life-long continence, repeated injections are required (Byron et al., 2011).

No major complications, meaning patients requiring revision surgery, have been reported. Minor complications, usually of transient nature, occur in approximately 15% of patients that underwent urethral bulking and include stranguria, hematuria, and vaginitis (Barth et al., 2005; Lüttmann et al., 2019).

Table 2. Published results of the surgical treatment of refractory incontinence with an artificial urethral sphincter.

	n	Follow-up (months)	Short-term continence		Long-terme continence		AUS cuff inflation	Complications cations		Owner satis-faction
			SX	SX + MED	SX	SX + MED		Minor	Major	
Rose et al. (2009)	4	27	75%	N/A	100%	-	75%	75%	0	100%
Delisser et al. (2012)	11	14	27%	N/A	36%	-	55%	82%	0	73%
Currao et al. (2013)	18	32	N/A	N/A	56%	77%	67%	44%	17%	N/A
Reeves et al. (2013)	27 (3M)	13	N/A	N/A	N/A	N/A	56%	37%	7%	89%
Gomes et al. (2018)	20	40	35%	-	35%	40%	65%	65%	5%	90%
Morgan et al. (2018)	9 (2M)	27	N/A	N/A	28%	N/A	71%	14%	14%	N/A

Short-term continence: < 6 months; long-term continence: > 6 months, SX: surgery, SX + MED: surgery and medication, N/A: data not available in the publication, M: male dogs. Only data from female dogs was evaluated when possible.

Transobturator vaginal tape

The technique was first described in women by Cho et al. (2011). Its most recent modification, the transobturator vaginal tape inside-out variant, is currently used in veterinary medicine (Claeys et al., 2010a; Hamon et al., 2019) (Figure 4). A polypropylene tape is applied around the distal third of the urethra using a specific needle and polyethylene tubes as a guide (Claeys et al., 2010a). The exact mechanism of action remains unclear, but increased periurethral tension due to the foreign body or an inflammatory response induced by the device might be the reason for regaining continence (Hamon et al., 2019).

In a study by Claeys et al. (2010a) including seven dogs, a complete continence was observed in 86% of patients after a mean follow-up of eleven months (Claeys et al., 2010a) (Table 1). Although Deschamps and Roux (2015) reported a complete urinary continence in eleven out of twelve dogs (92%) within two weeks after surgery, long-term results revealed complete long-term continence in only 25% of the dogs and an additional 25% of patients with major improvement at a median follow-up of four years. In a more recent study by Hamon et al. (2019), more positive results were reported, with complete continence in four out of ten dogs at a median follow-up of seven years (n = 12 dogs). However, two dogs in that study were excluded from the long-term analysis due to factors outside of the study's control. Additional medical treatment consisted of phenylpropanolamine, oestrogen or a combination of both. Due to this additional treatment, the continence rates of four out of the six remaining dogs that were not responding to solo treatment, improved (Hamon et al., 2019).

In a study by Deschamps and Roux (2015), major complications, such as fistula formation, occurred in three out of twelve dogs (25%) and required implant removal; however these complications did not occur in the two other studies (Claeys et al., 2010; Hamon et al., 2019). Minor complications occurred in 14-58% of patients and included transient dysuria and an iatrogenic urethral tear (Claeys et al., 2010; Deschamps and Roux, 2015; Hamon et al., 2019).

Artificial urethral sphincter

The artificial urethral sphincter (AUS) consists of an inflatable silicone cuff placed around the urethra that is connected to a subcutaneous access port (Adin et al., 2004) (Figure 5). The device is available in different sizes depending on the diameter of the urethra, which is measured either preoperatively via ultrasonographic examination or intraoperatively.

Tapered cuff inflation with sterile saline, adapted to the individual patient, is a unique advantage compared to other surgical techniques. In approximately 25-50% of patients, continence is regained due to the semi-rigid structure of the cuff and the more intra-abdominal position of the bladder (Rose et al., 2009). However, in dogs that remain incontinent four to six weeks postoperatively, inflation of the silicone cuff with 0.1–0.2 ml increments is performed (Reeves et al., 2013). According to a cadaveric study performed by Adin et al. (2004), the minimum volume to obtain complete occlusion of the lumen of the AUS cuff is 0.4 ml. Interestingly, recent studies show that higher total cuff inflation volumes of up to 2 ml might be required to achieve continence and can be tolerated by patients without adverse effects (Gomes et al., 2018; Morgan et al., 2018). In the event of side effects, such as stranguria or pollakiuria, the cuff can easily be deflated via the subcutaneous access port (Rose et al., 2009; Delisser et al., 2012; Currao et al., 2013; Gomes et al., 2018; Morgan et al., 2018).

Long-term continence rates in the literature vary between 28-100% (Rose et al., 2009; Delisser et al., 2012; Currao et al., 2013; Gomes et al., 2018; Morgan et al., 2018) (Table 2). When interpreting these results and comparing them with other surgical techniques, it is important to note that dogs with major improvement are not included in the continence rates. Delisser et al. (2012) for example found that 36% of the eleven dogs in their study were completely continent at the end of

the study. However, the median continence score of all dogs in that study was 9/10 (range, 7/10-10/10). In a recent study by Gomes et al. (2018) with a follow-up of three years (range: 365-2.257 days), 40% of complete continence and another 50% with major improvement (n = 20 dogs) were shown. In other studies, similar continence scores have been reported (Delisser et al., 2012; Currao et al., 2013; Gomes et al., 2018; Morgan et al., 2018) and scores at two weeks, three and six months were 8 (4 to 10). Another important factor in the evaluation of the results of the AUS system is the owner compliance (Currao et al., 2013). Final continence results may be lower due to owners deeming the situation clinically manageable and declining further follow-up visits to fill the cuff until full continence is achieved (Reichler et al., 2006; Currao et al., 2013; Byron, 2015). Currao et al. (2013) reported a long-term complete continence rate of 56% in dogs approximately 2.5 years after placement, which increased to 77% in cases with compliant owners allowing continued cuff inflations (Table 2).

Additional medical therapy should be considered when patients have recurrence of incontinence and have the cuff filled. Previous reports show a lot of variation in the need for additional medication, from 0-61% (Delisser et al., 2012; Currao et al., 2013). The infrequent requirement of medical therapy after AUS placement is likely a result of the semi-rigid structure of the cuff, as mentioned by Rose et al. (2009).

Major complications, including urethral obstruction, urethral stricture, urethral laceration, implant failure by cuff leakage, or implant infection have been reported in 0-17% of cases (Rose et al., 2009; Delisser et al., 2012; Currao et al., 2013; Reeves et al., 2013; Gomes et al., 2018; Morgan et al., 2018). Treatment consists of device removal (Currao et al., 2013). Minor complications are common (Delisser et al., 2012) and include dysuria, seroma formation and pain originating from the subcutaneous port. These complications are either transient or can be resolved with medications such as non-steroidals. Other minor complications such as stanguria require cuff deflation (Rose et al., 2009; Delisser et al., 2012; Currao et al., 2013; Reeves et al., 2013).

CONCLUSION

Evaluation of the current literature shows that there is a large disparity between results. Comparison is therefore not always accurate. The AUS system provides the best long-term results for the surgi-

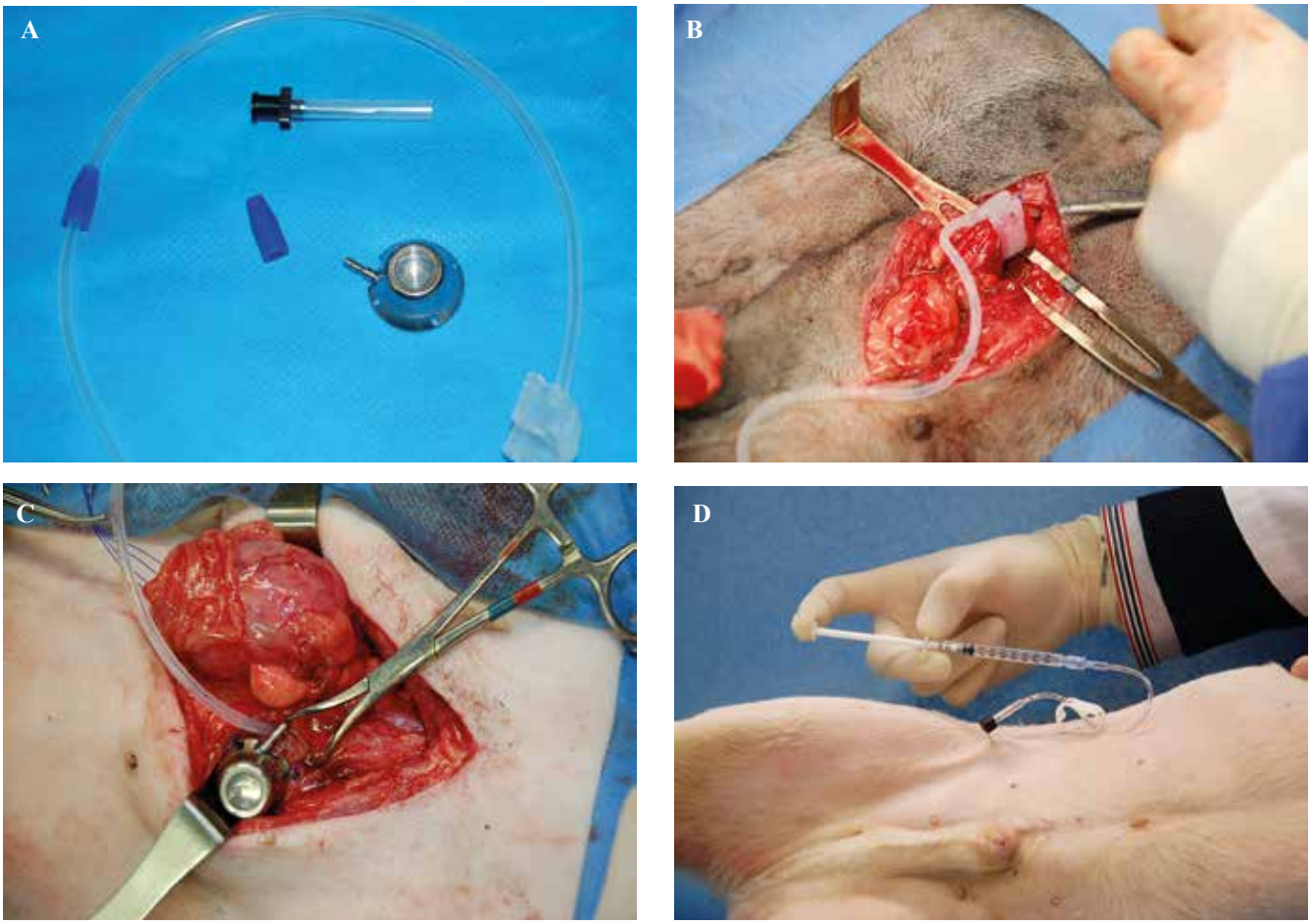

Figure 5. Placement of an AUS. A. An overview of the individual components. B. The inflatable cuff is placed around the urethra distal to the trigone. C. The subcutaneous access port is placed in a subcutaneous pocket lateral to the mammary chain. D. Postoperative filling of the cuff with sterile saline using a dedicated needle.

cal treatment of bitches with refractory UI, with most dogs achieving full continence. Cuff inflation allows individualized tapering until complete continence occurs, although only 55-75% of patients will require inflation to achieve complete urinary continence. For dogs that do not achieve full continence, supplementary medical therapy can further improve the continence rates.

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Gynaecologie: geen geschikte term in de diergeneeskunde

Het woord ‘gynaecologie’ wordt dikwijls gebruikt als synoniem voor verloskunde. Maar die tak van de (dier)geneeskunde is uiteraard veel ruimer. Dat weet iedereen. Etymologisch betekent gynaecologie kennis (Grieks: logos) over de huisvrouw (Grieks gynè: vrouw; oikos: huis). De term verraadt dat eeuwenlang enkel het geslachtsleven en de daarmee gepaard gaande ziektetoestanden van de huisvrouw - de getrouwde vrouw - voor de medische wetenschap van belang waren. ‘Geestelijke (geest-gelijke) dochters’, religieuzen, werden niet verondersteld een geslachtsleven te hebben en andere vrouwen waren uitschot, tenzij ze, bij voorkeur als jonge maagd, konden ‘dienen’ om huisvrouw te worden.
Gynaecologie: niet echt een geschikte term dus voor … diergeneeskundig gebruik. Maar welke dan wel?

Luc Devriese

LEEFTIJDSBEPALING BIJ DE HOND

Laatste oproep voor medewerking:

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Q-KOORTS EN DE GEVOLGEN VOOR DE VRUCHTBAARHEID BIJ HERKAUWERS

VRAAG

"Op een melkveebedrijf zonder duidelijke abortusproblemen maar met minder goede vruchtbaarheidsresultaten werden via tankmelk antistoffen tegen* Coxiella burnetii *aangetroffen. Zijn deze antistoffen van belang met betrekking tot de vruchtbaarheidsproblemen en is het nuttig om met vaccinatie te starten?"

ANTWOORD

Coxiella burnetii is een wereldwijd verspreide, obligaat intracellulaire, gramnegatieve bacterie die Q-koorts veroorzaakt (López-Helguera et al., 2013). Q-koorts is een zoönose en – hoewel vaak asymptomatisch – zijn de mogelijke klinische gevolgen van een infectie bij de mens duidelijk beschreven (Parker et al., 2006). Bij gedomesticeerde herkauwers, die als de belangrijkste infectiebron voor de mens worden beschouwd, is het pathologisch belang van Q-koorts minder duidelijk, aangezien de infectie vaak symptoomloos verloopt. Indien er toch een klinisch verloop is, uit Q-koorts bij schapen en geiten zich meestal onder de vorm van abortus (Arricau-Bouvery en Rodolakis, 2005; Sánchez et al., 2006). Hoewel de prevalentie bij koeien hoog is (in een studie van Van Praet (2019) bleek in Vlaanderen 81% van de onderzochte tankmelkstalen seropositief), verloopt Q-koorts bij runderen nog frequenter dan bij kleine herkauwers asymptomatisch (Ortega-Mora, 2012). De meest gerapporteerde klinische symptomen bij melkkoeien zijn voortplantingsstoornissen, zoals placentitis, abortus, doodgeboorte, het ophouden van de nageboorte, metritis en onvruchtbaarheid, en daarnaast mastitis (To et al., 1998; Bildfell et al., 2000; Barlow et al., 2008; García-Ispierto et al., 2010; López-Gatius et al., 2012). In andere studies kon dit verband tussen een *C. burnetii*-infectie en de hierboven genoemde reproductieproblemen echter niet aangetoond worden (Muskens et al., 2011; Agerholm, 2013). Garcia-Ispierto et al. (2013) vonden zelfs dat *C. burnetii*-seropositieve uitscheiders een korter interval partus-eerste tocht (dus snellere hervatting van de ovariële activiteit) en een korter interval partus-conceptie vertoonden. Mogelijk zijn deze positieve gevolgen voor de vruchtbaarheid te verklaren doordat eerder besmette seropositieve dieren immuun zouden zijn voor de gevolgen van een nieuwe (sub)klinische infectie of voor een heropflakkering tijdens perioden met een verlaagde immuniteit zoals de peripartumperiode.

Uit bovenstaande blijkt dus dat het aantonen van *C. burnetii* op een rundveebedrijf niet (steeds) wil zeggen dat dit ook een negatief effect heeft op de vruchtbaarheid. Mogelijk kan de controverse hierrond verklaard worden doordat er verschillende genotypes van de bacterie zijn, met eventueel een verschillend klinisch beeld (Jado et al., 2012).

Bovendien is de interpretatie van serologische tankmelkanalyse voor Q-koorts niet eenvoudig. Toch blijkt uit onderzoek van Taurel et al. (2012) dat bij een negatieve of laag-positieve ELISA-test op tankmelk een lage binnenbedrijfsprevalentie kan verwacht worden. Daarnaast blijkt er een significant verband te bestaan tussen het aantal doodgeboorten op een melkveebedrijf en het vinden van *C. burnetii*-antistoffen in de tankmelk (OR 1.484, dus 48% meer kans op een verhoogd aantal doodgeboorten wanneer de tankmelk seropositief is) (Ryan et al., 2018). Vermoedelijk is dit het gevolg van het feit dat *C. burnetii* een mogelijke oorzaak van abortus is. Daarnaast is het zo dat een *C. burnetii*-verwerping meestal gepaard gaat met een verhoogde uitscheiding van de bacterie en dus ook met een sterke spreiding van de infectie (Ryan et al., 2018). Desalniettemin is tankmelkonderzoek vooral geschikt voor epidemiologische monitoring. Om op bedrijfsniveau in te schatten of Q-koorts een probleem vormt, zijn echter extra analysen noodzakelijk (Piñero et al., 2014). Volgens Sidi-Boumedine et al. (2010) kan pas van een actieve *C. burnetii*-infectie gesproken worden wanneer een bedrijf tegelijk aan drie criteria voldoet, namelijk dat het te maken heeft met abortus of vruchtbaarheidsproblemen, dat de binnenbedrijfsprevalentie minstens 50% is en dat *C. burnetii*-DNA is aangetoond in vaginale swabs, nageboorte of een verworpen foetus.

Op basis van bovenstaande gegevens kan besloten worden dat een tankmelkonderzoek een eerste goedkope analyse kan zijn om blootstelling aan een mogelijke recente infectie in beeld te brengen. Dit tankmelkonderzoek kan daarbij het beste gecombineerd worden met serologisch onderzoek van een beperkt aantal vaarzen dat recentelijk bij de groep van de lacterende koeien werd gevoegd. Wanneer deze vaarzen seropositief zijn, wijst dit op een recente actieve infectie. Een volgende stap om een actieve infectie te bevestigen, is PCR-onderzoek van de placenta of vaginale mucus van vaarzen en koeien met reproductieproblemen (Sidi-Boumedine et al., 2010).

Q-koorts kan preventief aangepakt worden via vaccinatie. In België is voor geiten en runderen enkel een geïnactiveerd fase-I-vaccin tegen *C. burnetii* beschikbaar. Bij melkkoeien die op een besmet bedrijf aanwezig zijn, brengt vaccinatie een Th2-immuunreactie op gang en verkleint de kans op het uitscheiden van de kiem met een factor 5 in vergelijking met niet-

gevaccineerde dieren. Dit effect werd enkel vastgesteld bij naïeve (seronegatieve en PCR-negatieve), niet-drachtige dieren, maar is niet aangetoond na vaccinatie van naïeve, drachtige dieren (Guatteo et al., 2008). López-Helguera et al. (2013) toonden echter wel aan dat vaccinatie van seronegatieve, hoogproductieve melkkoeien tussen dag 171 en dag 178 van de dracht, een positief effect heeft op de vruchtbaarheid in de volgende lactatie in vergelijking met niet-gevaccineerde dieren. Een mogelijke verklaring voor dit positieve effect van vaccinatie op de vruchtbaarheid is dat vaccinatie seronegatieve dieren beschermt tegen de gevolgen van een latere infectie met *C. burnetii*. Het kan echter even goed zijn dat het vaccin een niet-specifieke immunostimulatie bij (hoog)drachtige koeien veroorzaakt die gunstig is voor de algemene gezondheid en dus ook voor de vruchtbaarheid van het dier. Opmerkelijk is dat dit positieve effect van vaccinatie op de vruchtbaarheid tijdens de dracht afwezig is bij seropositieve koeien. Aangezien vaarzen meestal nog seronegatief zijn (Waag, 2007), zijn dit de meest geschikte kandidaten om gevaccineerd te worden voordat ze bij de lacterende koeien worden gehuisvest. Op een bedrijf met een lage seroprevalentie kan vaccinatie van zowel vaarzen als lacterende koeien eveneens zinvol zijn om de ziekte op korte termijn te eradiceren op bedrijfsniveau, zeker wanneer dit gebeurt in combinatie met het opsporen en opruimen van seropositieve dieren. Om het aantal uitscheiders, de omgevingsbesmetting en het aantal verwerpingen op een besmet bedrijf te verminderen, zou het volgens een model van Courcoul et al. (2011) zelfs effectiever zijn om zowel de koeien als de vaarzen te vaccineren. Op bedrijven met een hoge prevalentie moet vaccinatie uiteraard langer aangehouden worden om zo op langere termijn naïeve, jonge dieren te beschermen (Courcoul et al., 2011).

Vermeldenswaardig is het feit dat vaccinatie tegen *C. burnetii* bij lacterende koeien vaak gepaard gaat met een daling in de melk de eerste week na de enting. Deze productiedaling is het meest uitgesproken bij naïeve, seronegatieve dieren (Schulze et al., 2015). Een ander belangrijk punt is dat er geen DIVA-vaccins (i.e. "differentiating infected from vaccinated animals") tegen *C. burnetii* bestaan. Hierdoor is individuele of tankmelkserologie na vaccinatie niet langer bruikbaar om de bedrijfsstatus te monitoren.

Concluderend kan gesteld worden dat Q-koorts bij melkvee vaak subklinisch verloopt. De beschreven klinische symptomen komen vooral neer op abortus en een verminderde vruchtbaarheid. Mogelijk zijn er verschillende stammen, waardoor het uiteenlopende klinische beeld kan verklaard worden. Serologisch tankmelkonderzoek is geschikt voor epidemiologische monitoring, maar om in te schatten of een infectie op bedrijfsniveau al dan niet actief is, zijn extra individuele analysen noodzakelijk. Vaccinatie kan onder bepaalde omstandigheden preventieve bescherming bieden, maar de vraag dient gesteld te worden of de kosten die hieraan verbonden zijn opwegen tegenover de (soms beperkte of afwezige) klinische verschijnselen.

Dr. H. Van Loo
Vakgroep Voortplanting, Verloskunde, Bedrijfsdiergeneeskunde,
Faculteit Diergeneeskunde, Salisburylaan 133,
B-9820 Merelbeke

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Kat wurgen en opeten

(Gent, jaren 1850-1860)

Vader deed wat hij kon om ons eten te bezorgen. Zo had hij eens een schreiende kat in een keldergat gezien, het beest er uit getrokken en mee gebracht. “Weeg dat eens er zit twee kilo vlees aan. Hou ze een beetje vast.” Middelerwijl zocht hij een koord, maakte er een strop van en trok die over het beest zijn nek. “Hou de staart goed vast, ik zal de strop toehalen.”. De kat poogde te spartelen en haalde daardoor vanzelf de strop toe. Na een paar minuten was ze gewurgd. Onder de kinderen die nog niet te bed waren begonnen er te schreien uit medelijden met het arme beest, maar vader zei “Morgennoen zult ge niet schreeuwen”.

Terwijl de kat nog warm was, werd het hoofd er afgesneden en het vel afgestroopt, dan werd ze voor de nacht buiten het venster gehangen. Daarna ging vader het vel verkopen bij een poeldenier, die er hem een half frankske voor gaf. “Dat is voor het vet om ze mee te stoven,” zei hij. De volgende dag ging de neus van allen ter kermis, en het was alsof we aan een bruiloftsmaal zaten. In vele maanden hadden we zo niet gesmuld. “Ge ziet wel”, zei vader, “dat Onze Lieve Heer ons niet vergeet.” Waarop moeder antwoordde: “ik geloof niet dat Onze Lieve Heer zich met ons bemoeit, want als hij ons wilde helpen, zou hij ons wat anders zenden dan een kat.”

Van dan af ging vader alle avonden op kattenjacht, maar de vangst was gering: gedurende de hele winter drie of vier.

Naschrift. Fragment uit de in 1924 opgeschreven en in 1986 uitgegeven jeugdherinneringen van de Gentse kleermaker Pol De Witte (1848 – 1929). Deze rebelse socialist, die in conflict kwam met de partijbonzen, groeide op in de wijk Batavia vol dicht opeengepakte woningen voor het ‘werkvolk’. Die moesten plaats ruimen voor de universitaire complexen van ‘de Platteau’ en ‘de Rozier’. De titel van de memoires *Alles is omgekeerd* wil beklemtonen hoe sterk de levensomstandigheden en de mentaliteit van de mensen veranderden. Iets wat overduidelijk blijkt uit bovenstaand verhaal.

Uit: Pol De Witte: *Alles is omgekeerd. Hoe de werklieden vroeger leefden - 1848-1918*. Kritak, Leuven, 1986, p. 87.

Luc Devriese

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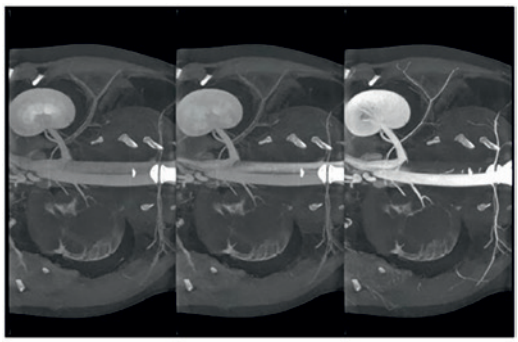

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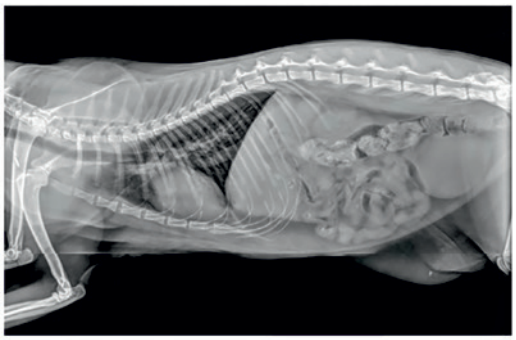

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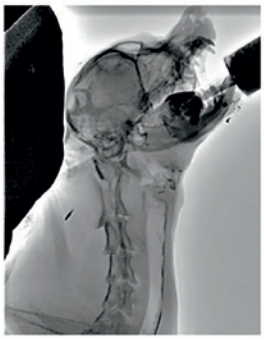

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BAYER