# Vlaams Diergeneeskundig Tijdschrift

FACULTEIT DIERGENEESKUNDE
accredited by EAEVE

SEPTEMBER-OKTOBER 2020
VOL. 89 - NR. 5

VERSCHIJNT TWEEMAANDELIJKS
PUBLISHED BIMONTHLY
ISSN 0303 9021
HTTPS://OJS.UGENT.BE/VDT
WWW.VDT.UGENT.BE
GENT X

Afgiftekantoor 9099 Gent X
v.u. Luc Peelman
Salisburylaan 133, B-9820 Merelbeke

UNIVERSITEIT GENT

# Wees ook nu extra alert voor hackers!


## Ontdek het op kbc.be/ondernemen/cyberveiligheid

KBC

# VLAAMS DIERGENEESKUNDIG TIJDSCHRIFT

**2020, vol. 89, nr. 5**


**INHOUD**



**Coverfoto: Christine Dudgeon**

Voor duikers is er vaak geen mooier noch rustgevender plek op aarde dan onder het wateroppervlak, hetzij in een aquarium, hetzij in open waters. Wanneer men plots oog in oog komt te staan met *een haai*, kan het echter niet anders dan dat adrenaline door de aderen raast. Deze coverfoto laat zien hoe een wetenschapper een zebrahaai rustig krijgt door deze letterlijk met een handomdraai in een omgekeerde positie te houden. De spieren van de haai verslappen en de haai kan enkele ogenblikken gewillig worden onderzocht en – na correctie van de houding – opnieuw wegzwemmen. Het enigma van tonische immobiliteit bij de haai wordt beschreven in dit nummer (pg. 243).

Tekst: Annelies Declercq

# VLAAMS DIERGENEESKUNDIG TIJDSCHRIFT
# ISSN 0303-9021
# HTTPS://OJS.UGENT.BE/VDT

**Hoofdredacteur en verantwoordelijke uitgever:** Luc Peelman
**Coördinator en eindredacteur:** Nadia Eeckhout
**Redacteur rubriek "Uit het verleden"**: Luc Devriese

**Redactiecomité:**
P. Bols, B. Broeckx, C. Burvenich, E. Cox, S. Daminet, W. De Spiegelaere, M. Devreese, L. Devriese, R. Ducatelle, M. Haspeslagh, M. Hesta, K. Houf, B. Pardon, I. Polis, J. Saunders, L. Van Ham, F. Van Immerseel, A. Van Soom

**Druk:** Graphius
Eekhoutdriesstraat 67, B-9041 Oostakker

**Publiciteit:**
Boerenbond – Mediaservice, Diestsevest 40, B-3000 Leuven
Tel. 016 28 63 33

**Inlichtingen (voor auteurs) en Abonnementen:**
Nadia Eeckhout
Salisburylaan 133, B-9820 Merelbeke
Tel. 09 264 75 13
nadia.eeckhout@UGent.be

**Editor-in-chief and publisher:** Luc Peelman
**Editorial office:** Nadia Eeckhout
**Editor "History":** Luc Devriese

**Editorial board:**
P. Bols, B. Broeckx, C. Burvenich, E. Cox, S. Daminet, W. De Spiegelaere, M. Devreese, L. Devriese, R. Ducatelle, M. Haspeslagh, M. Hesta, K. Houf, B. Pardon, I. Polis, J. Saunders, L. Van Ham, F. Van Immerseel, A. Van Soom

**Printed by:** Graphius
Eekhoutdriesstraat 67, B-9041 Oostakker

**Advertisements:**
Boerenbond – Mediaservice, Diestsevest 40, B-3000 Leuven
Tel. 016 28 63 33

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

Het Vlaams Diergeneeskundig Tijdschrift verschijnt 6 maal per jaar en wordt uitgegeven door de Faculteit Diergeneeskunde, Universiteit Gent.
Voor intekening dient U contact op te nemen met het secretariaat van het tijdschrift: nadia.eeckhout@UGent.be; tel. 09 264 75 13; fax 09 264 77 99. Er zal u een factuur toegestuurd worden van 60 euro (+6% BTW) (abonnees in België) of 80 euro (+6% BTW) (abonnees in het buitenland). Studenten en faculteitspersoneel kunnen genieten van een gunsttarief.

De verantwoordelijkheid voor alle gepubliceerde methoden, materialen en aanbevelingen berust bij de auteurs van de betreffende bijdragen. De redactie en uitgever zijn niet verantwoordelijk voor eventuele letsels of schade als gevolg van toepassingen die daaruit voortvloeien.

The 'Vlaams Diergeneeskundig Tijdschrift' is published six times per year by the Faculty of Veterinary Medicine, Ghent University. For subscriptions, please contact the administrative offices of the journal: nadia.eeckhout@UGent.be; tel. 0032 9 264 75 13; fax 0032 9 264 77 99. An invoice of 80 euros (+6% VAT) will be sent.

The responsibility for all methods, materials and recommendations published herein rests solely with the authors of the various contributions. No responsibility is assumed by the editorial staff or publisher for any resulting injury or damage.

More detailed information is available on
HTTPS://OJS.UGENT.BE/VDT


**Beknopte richtlijnen voor auteurs**

Ieder manuscript zal qua inhoud en vorm beoordeeld worden door 2 onafhankelijke personen.
De samenvatting mag niet langer zijn dan 5% van het artikel met een max. van 150 woorden.
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.
In de **literatuurlijst** dienen achtereenvolgens vermeld: namen van auteur(s), initialen van voornamen, jaartal, titel van artikel, naam van tijdschrift, volume, paginering. Voorbeeld: Allan W.R., Rowson L.B., (1973). Control of the mare's oestrus cycle by prostaglandins. *Journal of Reproduction and Fertility 33*, 539-543.
De referenties zijn alfabetisch gerangschikt. Artikels van dezelfde auteur(s) dienen per jaartal gerangschikt en in de tekst aangeduid te worden als: (1975a, 1975b)… Bij boeken dienen plaats en naam van uitgever vermeld te worden.

**Figuren en tabellen** dienen contrastrijk te zijn en op afzonderlijke bijlagen te worden ingediend. De figuren moeten een grootte hebben van minstens 200 kb.
Het aantal tabellen en figuren wordt tot een noodzakelijk minimum beperkt.
Voor de figuren dienen titels en teksten gezamenlijk op een apart blad aangebracht te worden.

**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.

Verdere details kunnen verkregen worden op de redactie of op www.vdt.ugent.be

# "Fight, flight or freeze" Tonische immobiliteit bij haaien

*Fight, flight or freeze*
*Tonic immobility in sharks*

**[1]E. De Swaef, [1]M. Vercauteren, L. Pieraets, A. M. Declercq**

Vakgroep Pathologie, Bacteriologie en Pluimveeziekten, Faculteit Diergeneeskunde, Universiteit Gent, Salisburylaan 133, 9820 Merelbeke, België
[1]gedeelde eerste auteur

andclerc.declercq@ugent.be

## ABSTRACT

**Tonische immobiliteit (TI) is een niet-aangeleerde en omkeerbare toestand van motorische onbeweeglijkheid die bij verscheidene terrestrische en aquatische diersoorten, zoals haaien, kan worden uitgelokt. TI is van oorsprong een natuurlijk gedrag dat bij haaien kan worden uitgelokt door plaatselijke druk uit te oefenen ter hoogte van de vinnen en de dieren op de rug te draaien, waarna ze een verslapping van de spiertonus vertonen en tijdelijk niet meer reageren op omgevingsstimuli. Deze toestand kan mogelijk een effect hebben op de fysiologie van de dieren. TI zou bij haaien de ventilatie-efficiëntie verminderen en bijgevolg verscheidene bloedparameters beïnvloeden, stressrespons induceren en de osmolariteit van het bloed verstoren. Desalniettemin kent TI bij haaien wetenschappelijke en diergeneeskundige toepassingen bij (klinisch) onderzoek, bloedafname en wondbehandeling. Tonische immobiliteit bij haaien is echter een enigmatisch fenomeen en verdient verder interdisciplinair onderzoek.**

## SAMENVATTING

Tonic immobility (TI) is an inherited and reversible status of motoric immobility that can be provoked in a variety of terrestrial and aquatic animal species, including sharks. In sharks, this behavior, which is originally natural behavior, can be induced by putting external pressure on the fins and turning the animal on its back, after which the sharks will display reduced muscle tone and will temporarily not respond to environmental stimulation. It can be expected that this state has an effect on the physiology of the animal. In sharks, it can induce lowered ventilation efficiency with impact on blood parameters, it can cause a stress response and disrupt the osmolarity of the blood. Nonetheless, this TI state in sharks has its applications in scientific and veterinary (clinical) research, blood sampling and wound treatment. However, tonic immobility in sharks remains an enigmatic phenomenon that should be studied in detail using an interdisciplinary approach.

## INLEIDING

Haaien behoren tot de klasse van de kraakbeenvissen of Chondrichthyes (Weigmann, 2016). De meeste haaien zijn predatoren, hoewel enkele soorten ook opportunistische aaseters of zelfs filtervoeders zijn (bijvoorbeeld de reuzenbekhaai (*Megachasma pelagios*)) (Camhi et al., 1998). Hoewel de meeste soorten toppredatoren zijn, kunnen haaien zelf ook ten prooi vallen aan onder meer orka's (*Orcinus orca*) (Hughes, 2009; Jorgensen et al., 2019). De jachttechniek van de orka's bestaat erin de witte haai (*Carcharodon carcharias*) uit te putten en vervolgens op het gepaste moment in de zij te beuken zodat de haai op de rug draait (Pyle et al., 1999; Hughes, 2009). De aanvaller houdt hierbij de omgedraaide haai voor een bepaalde

tijd vast, waardoor de haai in een soort van motorisch onbeweeglijke toestand of tonische immobiliteit (TI) gaat en de orka zijn prooi kan verorberen (Pyle et al., 1999; Hughes, 2009; Brooks et al., 2011). Verder zou TI een rol spelen in het paringsritueel bij haaien (Pratt en Carrier 2001; Kunze en Simmons 2004; Brunnschweiler en Pratt, 2008; Williamson et al., 2018). Tonische immobiliteit gaat gepaard met heel wat fysiologische veranderingen, waardoor deze toestand niet zonder gevaar is voor het dier in kwestie. Tonische immobiliteit blijft een van de minst bestudeerde en meest enigmatische fenomenen in de biologie van haaien en verdient verder interdisciplinair onderzoek (Brooks et al., 2011).

In dit artikel wordt een overzicht gegeven van de functie en fysiologie van TI bij haaien en de mechanismen die TI kunnen uitlokken. Hierbij worden de huidige hiaten in de kennis van TI in kaart gebracht. Verder worden bestaande biologische en diergeneeskundige toepassingen van TI in het onderzoeksdomein beschreven.

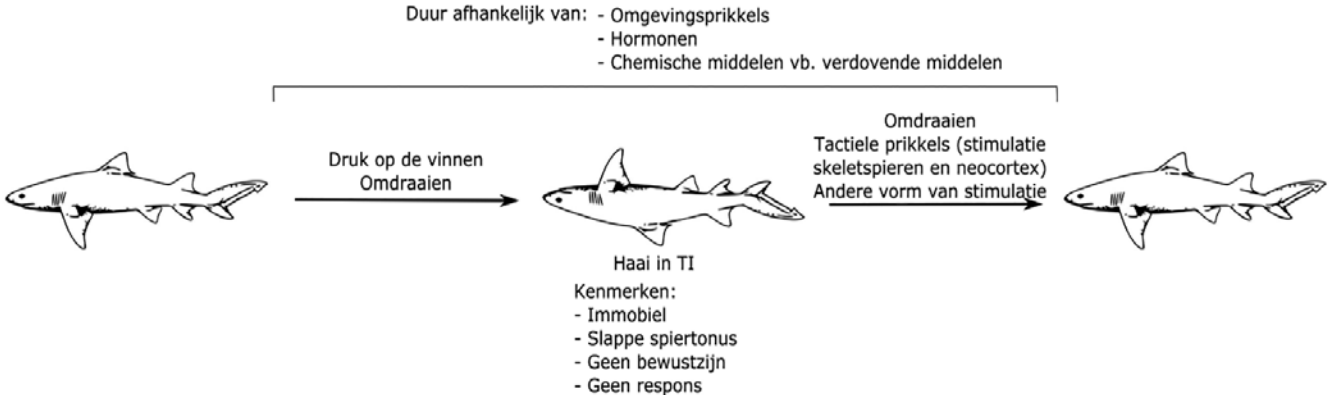
## TONISCHE IMMOBILITEIT EN UITLOKKENDE FACTOREN BIJ HAAIEN

Tonische immobiliteit (TI) is een niet-aangeleerde en omkeerbare toestand van motorische onbeweeglijkheid die ontstaat als reactie op een dreiging waaraan het dier niet meer kan ontsnappen (Volchan et al., 2011). In deze toestand wordt niet meer gereageerd op (schadelijke en pijnlijke) omgevingsprikkels (Hennig en Dunlap, 1976; Gallup, 1977; Thompson et al., 1981; Wells et al. 2005; Brooks et al., 2011; Williamson et al., 2018). Tonische immobiliteit komt voor bij verscheidene terrestrische en aquatische diersoorten, zoals konijnen (Ewell en Cullen, 1981), varkens (Erhard et al., 1999), kippen (Fogelholm et al., 2007) en goudvissen (*Carassius auratus*) (Richardson et al., 1977). Bij het merendeel van deze diersoorten speelt TI een rol in predator-prooi-interacties, waarbij de dieren (prooien) proberen op te gaan in hun omgeving en zo aan de aandacht van de predator te ontsnappen (Gallup, 1977; Watsky en Gruber, 1990; Henningsen, 1994; Brooks et al., 2011; Williamson et al., 2018). Ook bij de mens lijkt dit fenomeen te bestaan (Volchan et al., 2011) en werd dit beschreven als reactie op seksueel misbruik (Burgess en Holmstrom, 1976; Möller et al., 2007) en bij verschillende (natuur)rampen (Leach, 2004).

Met betrekking tot haaien werd TI reeds waargenomen bij verscheidene soorten, waaronder de citroenhaai (*Negaprion brevirostris*) (Watsky en Gruber, 1990; Rasmussen en Gruber, 1993; Murchie et al., 2009; Brooks et al., 2011), gladde hondshaai (*Mustelus canis*) (Whitman et al., 1986), witte haai (*Carcharodon carcharias*) (Pyle et al., 1999), zebrahaai (*Stegostoma tigrinum syn. S. fasciatum*) (Kunze en Simmons, 2004; Brunnschweiler en Pratt, 2008; Williamson et al., 2018), zwartpuntrifhaai (*Carcharhinus melanoptera*) (Davie et al., 1993), tijgerhaai (*Galeocerdo cuvier*) (Holland et al., 1999; Heithaus, 2001) en verpleegsterhaai (*Ginglymostoma cirratum*) (Pratt en Carrier, 2001). De exacte werking van dit complex, natuurlijk fenomeen is nog niet geheel opgeklaard (Benoff en Siegel, 1976; Hennig en Dunlap, 1976; Woodruff et al., 1976; Gallup, 1977; Gallup et al., 1980; Henningsen, 1994). Onderzoek naar de inductie en reversie van TI bij haaien werd tot nu toe bijna uitsluitend waargenomen of uitgevoerd in laboratoria of in een gecontroleerde omgeving, zoals in aquaria (Henningsen et al., 1994; Brooks et al., 2011). In het wild is het vaak moeilijk om haaien op te sporen, te volgen en hun natuurlijke gedrag te bestuderen, niet in het minst omdat heel wat soorten op grote dieptes leven en nachtactief zijn (Camhi et al., 1998). Bovendien kunnen niet alle soorten in laboratoriumcondities worden gehouden, wat resulteert in een bias in de huidige kennis die gebaseerd is op slechts een beperkt aantal soorten in experimentele situaties terwijl er een grote diversiteit aan haaien in de oceanen bestaat (Schluessel et al., 2008; Kempster et al., 2012).

Tonische immobiliteit bij haaien kan optreden door de dieren in de normale fysiologische beweging te beperken en/of door ze in een afwijkende houding te brengen (Gallup, 1977; Gallup et al., 1980; Watsky en Gruber, 1990; Davie et al., 1993). Dit kan door plaatselijke druk uit te oefenen ter hoogte van de rug- (Whitman et al., 1986), borst- of staartvin (Williamson et al., 2018) en het dier hierbij op de rug te draaien (Whitman et al., 1986; Watsky en Gruber, 1990; Henningsen, 1994) (Figuur 1). Bij het optreden van TI wordt het dier immobiel (Watsky en Gruber, 1990; Henningsen, 1994) en reageert het niet meer op schadelijke of pijnlijke stimulatie (Williamson et al., 2018). Verder zou TI bij haaien ook ontstaan door water in de kieuwkamer te pompen (Wells et al., 2005).

**Figuur 1. Voorbeeld van hoe een zebrahaai tijdens een duik in TI wordt gebracht door druk uit te oefenen op de staart (Foto: Christine Dudgeon).**




**Figuur 2. Schematisch overzicht van de kenmerken van TI en hoe TI geïnduceerd en omgekeerd kan worden. Bovendien worden enkele factoren vermeld die de duur kunnen beïnvloeden.**

In tegenstelling tot bij terrestrische dieren, ontspannen de spieren van aquatische dieren tijdens TI (Henningsen, 1994; Williamson et al., 2018). Bovendien vertraagt de ademhalingsfrequentie, wordt de ademhaling dieper en reageert het dier niet meer op omgevingsprikkels (Brooks et al., 2011).

TI treedt relatief snel op (in minder dan één minuut) (Whitman et al., 1986; Henningsen, 1994) en houdt minder dan een minuut tot uren aan bij haaien die niet verder worden vastgehouden (Watsky en Gruber, 1990; Henningsen, 1994). Tonische immobiliteit kan tevens onderbroken worden door de omgedraaide haai opnieuw in een normale houding te brengen (Henningsen, 1994). De inductie- en tijdsduur van de TI-toestand zijn individueel- en soortafhankelijk (Davie et al., 1993; Henningsen, 1994; Brooks et al., 2011; Williamson et al., 2018) (Figuur 2).

Het ontwaken uit TI kan versneld of vertraagd worden door bepaalde omgevingsstimuli, zoals geluid of een elektrische shock, maar ook door hormonen en chemische middelen (Hennig en Dunlap, 1976; Woodruff et al., 1976; Gallup, 1977). Zo wordt bij het gebruik van verdovende middelen een verlenging van de TI-duur waargenomen. Waarschijnlijk reduceren de verdovende middelen zowel angst alsook het cholinergisch systeem dat een mogelijke rol speelt bij TI-inductie (Woodruff et al., 1976; Gallup, 1977). Bij andere diersoorten kunnen visuele prikkels de duur van de TI-toestand beïnvloeden. Zo verlengt de nabijheid van een predator de duur van TI bij kippen (Gallup, 1977). Ook kunnen hormonen, zoals scopolamine en fysostigmine, een effect hebben op de duur van TI, hoewel hier in de literatuur uiteenlopende resultaten over worden beschreven (Woodruff et al., 1976).

## INVLOED VAN TONISCHE IMMOBILITEIT OP DE FYSIOLOGIE VAN HAAIEN

Er is weinig bekend over de effecten van TI op de fysiologie van haaien (Brooks et al., 2011; Williamson et al., 2018). Er wordt gesuggereerd dat TI weinig belijvende negatieve gevolgen induceert, gezien de lage spierspanning en diepe ritmische ventilatie (Watsky en Gruber, 1990). Indien waterstroom over de kieuwen wordt gegarandeerd, blijven bij zwartpuntrifhaaien bovendien de hartslag en bloeddruk behouden tijdens TI (Davie et al., 1993). Hoewel de dieren in een coma-achtige toestand verkeren, waarbij ze niet meer reageren op omgevings- en pijnprikkels (Hennig en Dunlap, 1976; Gallup 1977; Thompson et al. 1981; Wells et al. 2005; Brooks et al., 2011; Williamson et al., 2018), is het niet bekend of ze effectief pijn noch stress ondervinden (Brooks et al., 2011; Williamson et al., 2018). Tijdens TI verlaagt de ventilatiefrequentie, wat net zoals de respiratiefrequentie bij zoogdieren, gepaard gaat met veranderingen ter hoogte van het hart en de bloedsomloop (Davie et al., 1993; Brooks et al., 2011). Klemm (1971, 1976) beschreef de werking van een immobiliteitsreflex-controlesysteem ter hoogte van de hersenstam dat een inhiberende werking heeft op de skeletspieren bij konijnen. Het specifieke TI-werkingsmechanisme bij haaien is nog niet opgehelderd, maar werd wel reeds aangetoond bij ratten, konijnen en kikkers (Carli, 1971; Klemm, 1971; Klemm, 1976). De vraag blijft echter of de daling in ventilatie en hartslag een rechtstreeks effect is van TI volgens de theorie van Klemm (1976) of dat dit een gevolg is van het omdraaien van de dieren, en de anatomische inversie op die manier een bemoeilijkte ademhaling en veranderingen ter hoogte van het cardiorespiratoir stelsel induceert.

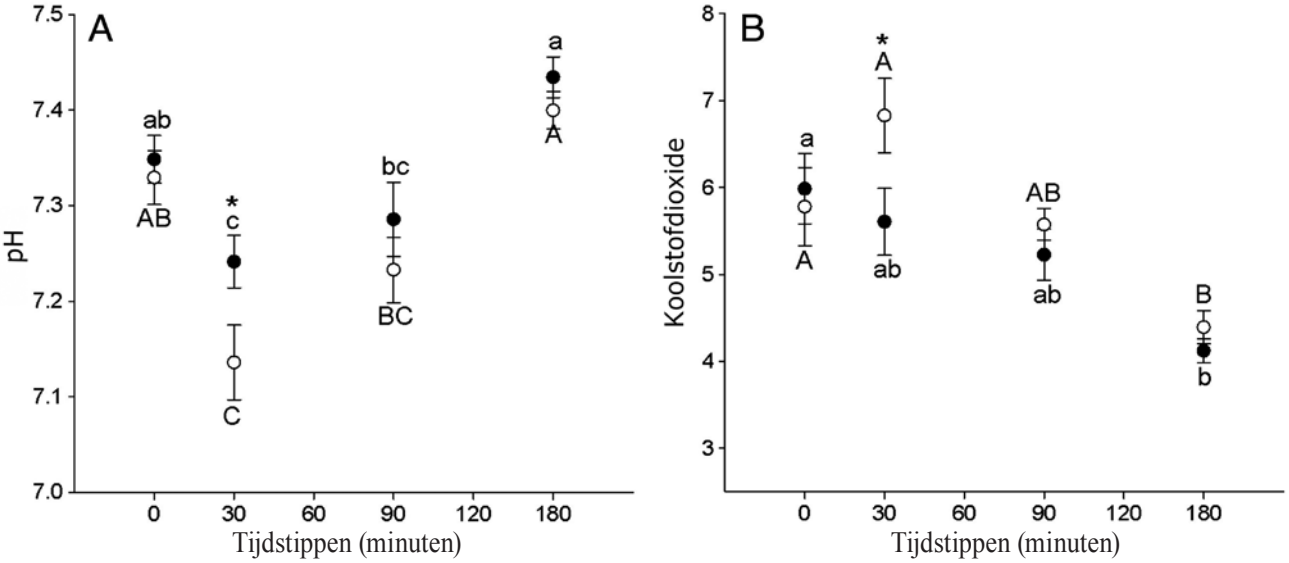
Tot op heden werd er slechts één studie uitgevoerd waarin de effecten van TI op de fysiologische homeostase bij elasmobranchen, meer bepaald jonge citroenhaaien, werden onderzocht (Brooks et al., 2011). In deze studie werden de bloedparameters van citroenhaaien die een half uur tot drie uur in TI-toestand werden gehouden, vergeleken met deze van een groep citroenhaaien die vrij konden zwemmen (niet in TI-toestand). Uit de resultaten van deze studie blijkt dat TI een stressvolle gebeurtenis is, waarbij verscheidene bloedparameters worden verstoord (Brooks et al., 2011). Fysiologische veranderingen in het bloed

kunnen worden opgedeeld in drie grote groepen: 1. pH- en koolstofconcentraties, 2. stresshormonen en 3. bloedosmolariteit.

In de studie van Brooks et al. (2011) vertoonden de citroenhaaien in TI in het eerste half uur een significante daling van de bloed-pH-waarde en een significante stijging van koolstofdioxideconcentraties in vergelijking met de soortgenoten die vrij konden zwemmen. Tonische immobiliteit bij jonge citroenhaaien verstoort bijgevolg de efficiëntie van de ventilatie op korte termijn (Brooks et al., 2011). Dit is waarschijnlijk te wijten aan de minder efficiënte manier van buccale pompventilatie tijdens TI, in tegenstelling tot ramventilatie die wordt uitgeoefend bij vrij zwemmen (Brooks et al., 2011): bij haaien kan een onderscheid gemaakt worden tussen twee ventilatietechnieken: buccale pompventilatie en ramventilatie (Carlson en Parsons, 2001). Buccale pompen zijn wangspieren die actief het water over de kieuwen pompen om zo voldoende zuurstof uit het water te kunnen onttrekken. Deze ventilatietechniek laat het dier toe gedurende een langere tijd in stilstand te blijven (Roberts, 1978). Ramventilatie is de passieve stroming van water over de kieuwen die ontstaat bij beweging van het dier. Sommige haaien, zoals de witte haai, zijn obligaat ramventilerend, waardoor deze nagenoeg continu in beweging moeten blijven (Carlson en Parsons, 2001; Klimley, 2013). De witte haai in TI-toestand kan als obligaat ramventilerend dier niet terugvallen op het buccaal pompen en kan het gebrek aan ventilatie bijgevolg niet compenseren. Zonder ingrepen, zoals kunstmatige ventilatie, zal zijn ademhaling na een tijd bijgevolg stilvallen (Brooks et al., 2011), wat tot de dood kan leiden. De meeste haaien, onder andere ook citroenhaaien, kunnen afhankelijk van hun activiteit beide ventilatietechnieken toepassen (Carlson en Parsons, 2001; Klimley, 2013), waarbij ramventilatie efficiënter zou zijn (Brooks et al., 2011). In de studie van Brooks et al. (2011) werden de significante verschillen in bloed-pH-waarde en koolstofdioxideconcentraties bij de finale staalname (na drie uur) genivelleerd, wat erop wijst dat citroenhaaien geslaagde compensatiemechanismen in werking stellen om gasuitwisseling te bevorderen (Figuur 3). Een van die compensatiemechanismen wordt in de verhoogde ventilatiesnelheid (via buccaal pompen) gezien bij dieren in TI. Een verhoogde ventilatiesnelheid tijdens TI zorgt voor een verhoogde passage van water over de kieuwfilamenten, wat de capaciteit van gasuitwisseling stimuleert (Butler en Metcalfe, 1989; Carlson en Parsons, 2003; Hawkins et al., 2004).

Naast de veranderingen in bloed-pH-waarde en koolstofdioxineconcentraties werden in de studie van Brooks et al. (2011) ook verhoogde bloedglucosewaarden gevonden bij dieren in TI-toestand, wat aangeeft dat de dieren chronische stress ondervonden.

In de laatste jaren is de interesse voor het onderzoek naar stressreacties bij aquatische dieren toege-




**Figuur 3. Variatie in bloedgasconcentraties (Y-as) bij jonge citroenhaaien (*Negaprion brevirostris*) als respons op het in bedwang houden van de dieren. De tijdstippen waarop de staalnames werden genomen zijn voorgesteld op de X-as. Volle punten (●) stellen de dieren voor die vrij konden zwemmen tussen de tijdstippen van de staalnames in en de lege punten (○) stellen de dieren voor die in tonische immobiliteit (TI) werden gehouden. De data stellen de gemiddelde bloedchemiewaarden voor van (A) pH en (B) koolstofdioxide. De asterisks (*) geven de statistisch significante verschillen aan tussen dieren in TI- en niet in TI-toestand op een bepaald tijdstip (gepoolde t-test, α=b0.05). Verschillen in hoofdletters geven statistische verschillen weer tussen de tijdstippen van staalname voor behandelingen waarbij TI werd aangehouden; verschillen in kleine letters voor deze waarbij geen TI werd toegepast (ANOVA, α=b0.05) (naar: Brooks et al., 2011).**

nomen; de kennis daaromtrent is bijgevolg nog zeer beperkt (Petitjean et al., 2019). Ook bij haaien werd nog maar weinig onderzoek uitgevoerd en al zeker niet in combinatie met TI. Concrete resultaten over hoe TI inspeelt op het stressmechanisme (of omgekeerd) ontbreken bij de haai, maar er werden reeds verscheidene studies uitgevoerd bij andere diersoorten. Onderzoek bij de kip toonde aan dat langdurige TI vergelijkbaar is met chronische stress, waarbij hoge concentraties stresshormonen (glucocorticoiden) worden vrijgesteld (Duan et al., 2014; Fu et al., 2014). Dit resulteert in de inhibitie van de proteïnesynthese en activatie van proteolyse ter hoogte van de skeletspieren. Bij langdurige TI kunnen een verandering in de samenstelling van actine-myosineketens, een vermindering van "heat-shock"-proteïnen en een verstoring van het glucosemetabolisme ontstaan (Fu et al., 2014). Dit heeft spieratrofie en vertraagde groei tot gevolg (Duan et al., 2014; Fu et al., 2014). Glucocorticoïden spelen ook bij haaien een cruciale rol in de primaire stressrespons, gezien ze door de metabolisatie van leverglycogeen een voldoende toevoer van energie verzekeren (Barton, 2002; Busch en Hayward, 2009; Skomal en Bernal, 2010). De primaire stressrespons wordt gekarakteriseerd door een cascade van catecholaminen en corticosteroïden die een reeks fysiologische en fysische adaptaties uitlokken om de "vecht-of-vluchtreactie" te activeren en de kans op overleving te vergroten (Romero, 2004; Busch en Hayward, 2009; Skomal en Bernal, 2010). Sommige van deze aanpassingen (zoals het openzetten van de kieuwbloedvaten) dienen om de kieuwperfusie en de capaciteit tot gasuitwisseling te stimuleren (Randall, 1982; Skomal en Bernal, 2010). Deze aanpassingen verhogen echter ook de ionenpermeabiliteit in de kieuwen, wat tot een verstoring van de elektrolytenbalans kan leiden (Randall, 1982; Gonzalez en McDonald, 1992). Deze imbalans resulteert in een continue diffusie van ionen via de kieuwen naar het bloed, wat door zoutexcretie van de rectale klier en de nieren wordt veroorzaakt (Shuttleworth, 1988). In de studie van Brooks et al. (2011) lokte TI bij citroenhaaien een verstoring van de elektrolytenbalans uit. De plasmawaarden van magnesium, natrium en calcium waren significant verhoogd, daar waar de kaliumwaarden net een significante daling kenden (Brooks et al., 2011). Dit wijst erop dat de verhoogde permeabiliteit van de kieuwen en de hormonale cascade die dit proces veroorzaakt, toch een belangrijk effect hebben tijdens TI.

Naast de bovengenoemde gevolgen heeft TI ook invloed op de spieren. Algemeen zorgt TI bij haaien voor een relaxerende spiertonus, dit in tegenstelling tot bij terrestrische dieren (Whitman et al., 1986; Henningsen, 1994; Williamson et al., 2018). Een belemmering van bepaalde tactiele en proprioceptieve functies, zoals druk ter hoogte van de vinnen en dorsale inversie, zou inhiberende spinale neuronen activeren en zo het motorisch systeem verlammen (Klemm, 1976). Tonische immobiliteit kan worden versterkt via het limbisch systeem dat in angstsituaties epinefrine vrijstelt (Klemm, 1976). Inhibitie van TI zou optreden door tactiele prikkels, die een activering van de neocortex en skeletspier veroorzaken (Klemm, 1976). Concrete aanwijzingen van hoe TI inspeelt op de spierwerking bij de haai ontbreken maar er werden wel reeds verscheidene studies uitgevoerd bij andere diersoorten (Klemm, 1976).

## FUNCTIES VAN TONISCHE IMMOBILITEIT

### Bescherming

Haaien kunnen ten prooi vallen aan orka's (Hughes, 2009; Jorgensen et al., 2019). De jachttechniek van orka's bestaat erin de haai uit te putten, op de rug te draaien en zo een bepaalde tijd vast te houden, waardoor de haai in een coma-achtige TI-toestand belandt (Pyle et al., 1999; Hughes, 2009; Brooks et al., 2011). Deze TI-toestand zou een finaal stadium van een defensieve cascade zijn als respons op de aanwezigheid van een predator (Ratner, 1967; Zlotkin en Gruber, 1984; Davie et al., 1993; Brooks et al., 2011; Williamson et al., 2018). De cascade zou starten met een periode van vrijwillige immobiliteit die bedoeld is om de kans om opgemerkt te worden, te verlagen. Daarna zou deze toestand overgaan in een "vecht-of-vluchtreactie", waarbij het niet kunnen vluchten het ontstaan van TI zou teweegbrengen (Marx et al., 2008), hetgeen in bovenstaand voorbeeld zoveel betekent als het fataal ten prooi vallen aan de orka's (Hughes, 2009). Haaien kunnen zelf uit TI ontwaken indien ze niet meer op de juiste manier in bedwang worden gehouden (Henningsen, 1994). Wanneer de situatie echter te lang wordt aangehouden, bijvoorbeeld door een orka tijdens de jacht, en de impact op hun fysiologie te groot wordt, kan TI tot sterfte leiden.

### Voortplanting

Tonische immobiliteit kan een belangrijke functie uitoefenen tijdens het paringsritueel bij haaien (Whitman et al., 1986; Davie et al., 1993; Williamson et al., 2018). Verschillende haaien, waaronder de zandbankhaai (*Carcharhinus plumbeus*), de citroenhaai en de hondshaai (*Scyliorhinus canicula)* zijn haaisoorten die inwendig bevruchten en TI gebruiken tijdens het paringsritueel (Whitman et al., 1986). Hierbij bijt het mannetje zich vast in de pectorale (bijvoorbeeld bij de verpleegsterhaai), caudale (bijvoorbeeld bij de zebrahaai) of dorsale (bijvoorbeeld bij de grote blauwe haai (*Prionace glauca*)) vin om het vrouwtje om te draaien. Eenmaal op haar rug, bereikt het vrouwtje TI en brengt het mannetje zijn claspers in de cloaca, waardoor de bevruchting kan plaatsvinden (Klimley, 2013). De exacte reden voor dit gedrag tijdens de paring is tot op heden niet achterhaald. De vrouw-

tjes ontwaken spontaan na de paring maar het is nog niet duidelijk wat het onderliggende mechanisme is. Waarschijnlijk is de tijdsduur waarin de haai in TI wordt gehouden van groot belang. Zolang de buccale pompventilatie dit aankan, blijft de vitaliteit van het dier gegarandeerd. Dieren die obligaat ramventilerend zijn, vertonen vermoedelijk een ander voortplantingsgedrag of gebruiken compenserende mechanismen (in beweging blijven tijdens copulatie indien deze door TI moet worden geleid). Dit fenomeen werd echter nog niet nader onderzocht bij haaien.

## TOEPASSINGEN VAN TONISCHE IMMOBILITEIT BIJ HAAIEN

### "Shark repellents"

Er is behoefte aan een goedkoop en effectief haaiwerend middel voor onder andere duikers, zwemmers en surfers. Bovendien is er nood aan een methode om bijvangst van haaien in de commerciële visserij te reduceren (Hart en Collin, 2015). In de twee gevallen kan het haaiwerend middel een verschillende vorm van eenzelfde technologie aannemen, maar beide zijn gebaseerd op de basisneurobiologie van de gevoelsorganen, i.e. zicht, gehoor, chemoreceptie (geur en smaak), en elektroreceptie (ampullae van Lorenzini) van de haaien en op de invloed ervan op hun gedrag (Hart en Collin, 2015). Onderzoek naar het ideale haaiwerend middel of "shark repellent" is volop in ontwikkeling. Idealiter houden "shark repellents" de haaien op afstand of dienen ze een aanval door een haai te voorkomen (Zlotkin en Gruber, 1984; Watsky en Gruber, 1990). Het gebruik ervan wordt belemmerd door de fysische beperkingen en technische en logistieke moeilijkheden om dergelijke producten of toestellen in een open, mariene omgeving in te zetten en door de onvoorspelbare interactie met een complex wezen als de haai (Hart en Collin, 2015). Bovendien is het onderzoek naar "shark repellents" voor wetenschappers niet zonder gevaar, zeker voor wat meer agressieve haaiensoorten betreft. Door de dieren in TI te brengen, wordt onderzoek vereenvoudigd. De efficiëntie van dergelijke haaiafstotende middelen wordt getest door de reactie van haaien te observeren wanneer ze in contact worden gesteld met het product, waarbij toxiciteit, ontwaken uit TI en eetinhibitie bij agressieve haaien worden nagegaan (Zlotkin en Gruber, 1984).

Er werden reeds verschillende "repellents" onderzocht (Hart en Collin, 2005). Als chemische "repellent" werden onder meer rottend haaienvlees en kopersulfaat (al dan niet gecombineerd als koperacetaat) getest (Springer, 1955; Hodgson en Mathewson, 1978). Verder werden toxines afgescheiden door vissen van het genus *Pardachirus* (pardaxines, mosesines en pavoninines) effectief bevonden tegen bepaalde haaiensoorten en konden eveneens haaien wekken uit een toestand van TI (Tachibana et al., 1984; Tachibana et al., 1985; Tachibana en Gruber, 1988). Pardaxinesecreties blijken echter thermolabiel en verliezen deels hun activiteit tijdens vriesdrogen (Primor en Zlotkin, 1975; Clark en George, 1979). Ook sodiumdodecylsulfaat (SDS) en lithiumdodecylsulfaat (LDS) blijken een sterke aversie bij verscheidene haaiensoorten te induceren (Hart en Collin, 2015). Het gebruik van dergelijke chemische middelen in een open mariene omgeving is echter niet gewenst. In recenter onderzoek naar haaiafstotende middelen wordt gefocust op componenten die een biologische relevantie voor haaien hebben, zoals chemische componenten uitgescheiden door natuurlijke vijanden (Hart en Collin, 2015). Zo werden kairomones (feromonen uitgescheiden door de Amerikaanse krokodil (*Crocodylus acutus*), een natuurlijke predator van de citroenhaai) (Rasmussen en Schmidt, 1992) en necromones (semiochemische stoffen uitgescheiden door dode dieren) (Yao et al., 2009; Stroud et al., 2014) succesvol ingezet om haaien uit TI te wekken. De uitdaging bij deze chemische "repellents" blijft echter het isoleren van stoffen die niet toxisch zijn en bovendien in lage concentraties effectief zijn, zodat ze nog steeds werkzaam blijven wanneer ze in het water terechtkomen (Hart en Collin, 2015).

Naast chemische "repellents" werd ook onderzoek gedaan naar elektrische en magnetische pulsen, visuele en akoestische "repellents" (Hart en Collin, 2015).

### Tonische immobiliteit bij haaien en de link met diergeneeskunde

Tonische immobiliteit wordt beschreven als een voor mens en haai veilige manier om haaien in bedwang te houden tijdens het diergeneeskundig onderzoek in dierentuinen, onderzoeksinstellingen en aquaria (Henningsen, 1994; Clayton en Seeley, 2019). Deze techniek kan worden gebruikt bij pijnloze procedures, zoals een algemeen klinisch onderzoek en bij ingrepen, zoals bloedafname en wondbehandeling (Zlotkin en Gruber, 1984; Henningsen, 1994; Williamson et al., 2018). TI werd ook uitgelokt voor het toedienen van dwangvoeding en vloeistoftherapie na een periode van lang vasten of ziekte bij haaien in gevangenschap (Henningsen, 1994).

Elke manipulatie kan stress veroorzaken, waardoor de kans op automutilatie, onder andere kieuwbeschadigingen en wonden, vergroot. Het gebruik van TI als anesthesiemiddel werd beschreven door Kessel en Hussey (2015) bij implantatieprocedures. Verder onderzoek naar deze methode zou ook diergeneeskundige handelingen bij haaien, zoals hoger beschreven, kunnen verbeteren. Bij tal van andere diersoorten werd reeds een verband tussen pijnreductie en TI vastgesteld (Baker et al., 2019).

Het blijft echter noodzakelijk de ventilatie bij haaien te controleren en te stabiliseren, opdat TI geen fatale afloop zou hebben. Na het onderzoek kan de TI

onderbroken worden door de haai terug om te draaien en/of los te laten (Henningsen, 1994).

## DANKWOORD

De auteurs willen in het bijzonder Christine Dudgeon bedanken voor het delen van Figuur 1.

## REFERENTIE

Baker B., Machin K., Schwean-Lardner K. (2019). When pain and stress interact: Looking at stress-induced analgesia and hyperalgesia in birds. *World's Poultry Science Journal 75 (3),* 457-468.

Barnett A., Redd K.S., Frusher S.D., Stevens J.D., Semmens J.M. (2010). Non-lethal method to obtain stomach samples from a large marine predator and the use of DNA analysis to improve dietary information. *Journal of Experimental Marine Biology and Ecology 393*, 188-192.

Barton B.A. (2002). Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. *Integrative Comparative Biology 42*, 517-525.

Baum J.K., Myers R.A., Kehler D.G., Worm B., Harley S.J., Doherty P.A. (2003). Collapse and conservation of shark populations in the northwest Atlantic. *Science 299*, 389-392.

Benoff F., Siegel P. (1976). Genetic analysis of tonic immobility in young Japanese quail (*Coturnix cotunix japonica*). *Animal Learning & Behaviour 4 (2),* 160-162.

Brooks E.J., Sloman K.A., Liss S., Hassan-Hassanein L., Danylchuk A.J., Cooke S.J., Mandelman J.W., Skomal G.B., Sims D.W., Suski C.D. (2011). The stress physiology of extended duration tonic immobility in the juvenile lemon shark, *Negaprion brevirostris* (Poey 1868). *Journal of Experimental Marine Biology and Ecology 409 (1-2),* 351-360.

Brunnschweiler J.M., Pratt H.L. Jr. (2008). Putative male–male agonistic behaviour in free-living zebra sharks, *Stegostoma fasciatum*. *Open Fish Science Journal 1*, 23-27.

Burgess G.H., Beerkircher L.R., Cailliet G.M., Carlson J.K., Cortés E., Goldman K.J., Grubbs R.D., Musick J.A., Musyl M.K., Simpfendorfer C.A. (2005). Is the collapse of shark populations in the northwest Atlantic Ocean and Gulf of Mexico real? *Fisheries 30*,19-26.

Burgess A.W., Holmstrom L.L. (1976). Coping behavior of the rapevictim. *American Journal of Psychiatry 133*, 413-418.

Busch D.S., Hayward L.S. (2009). Stress in a conservation context: a discussion of glucocorticoid actions and how levels change with conservation-relevant variables. *Biological Conservation 142*, 2844-2853.

Butler P.J., Metcalfe J.D. (1989). Cardiovascular and respiratory systems. In: Shuttleworth T.J. (editor). *Physiology of Elasmobranch Fishes*. Springer-Verlag, New York, p. 1-42.

Camhi M., Fowler S.L., Musick J.A., Bräutigam A., Fordham S.V. (1998). *Sharks and their Relatives - Ecology and Conservation*. IUCN/SSC Shark Specialist Group. IUCN, Gland, Switzerland and Cambridge, UK.

Carlson J.K., Parsons G.R. (2001). The effects of hypoxia on three sympatric shark species : physiological and behavioral responses. *Environmental Biology of Fishes 61 (4),* 427-433.

Carlson J.K., Parsons G.R. (2003). Respiratory and hematological responses of the bonnethead shark, *Sphyrna tiburo*, to acute changes in dissolved oxygen. *Journal of Experimental Marine Biology and Ecology 294*, 15-26.

Carli G. (1971). Sub-cortical mechanisms of rabbit hypnosis. *Archives Italiennes De Biologie 109*, 15-26.

Clark E., George A. (1979). Toxic soles, *Pardachirus marmoratus* from the Red Sea and *P. pavoninus* from Japan, with notes on other species. *Environmental Biology of Fishes 4 (2),* 103-123.

Clayton L., Seeley K.E. (2019). Sharks and medicine. In: Miller R.E., Lamberski N., Calle P.P. (editors). *Fowler's Zoo and Wild Animal Medicine Current Therapy.* Volume 9, Elsevier Inc. Missouri, p. 338-344.

Davie P.S., Franklin C.E., Grigg G.C. (1993). Blood pressure and heart rate during tonic immobility in the black tipped reef shark, *Carcharhinus melanoptera*. *Fish Physiology and Biochemistry 12(2)*, 95-100.

Duan Y., Fu W., Wang S., Ni Y., Zhao R. (2014). Effects of tonic immobility (TI) and corticosterone (CORT) on energy status and protein metabolism in pectoralis major muscle of broiler chickens. *Comparative Biochemistry and Physiology. Part A: Molecular & Integrative Physiology 169*, 90-95.

Dudgeon C.L., Lanyon J.M., Semmens J.M. (2013). Seasonality and site fidelity of the zebra shark, *Stegostoma fasciatum*, in southeast Queensland, Australia. *Animal Behaviour 85 (2),* 471-481.

Erhard H. W., Mendl M., Christiansen S. B. (1999). Individual differences in tonic immobility may reflect behavioural strategies. *Applied Animal Behaviour Science 64 (1),* 31-46.

Ewell A.H., Cullen J.M. (1981). Tonic immobility as a predator-defense in the rabbit (*Oryctolagus cuniculus*). *Behavioral and Neural Biology 31,* 483-489.

Fitzpatrick R., Abrantes K.G., Seymour J., Barnett A. (2011). Variation in depth of whitetip reef sharks: does provisioning ecotourism change their behaviour? *Coral Reefs 30*, 569-577.

Fogelholm J., Inkabi S., Höglund A., Abbey-Lee R., Johnsson M., Jensen P., Henriksen R., Wright D. (2019). Genetical genomics of tonic immobility in the chicken. *Genes 10*, 341.

Fu W., Duan Y., Wang S., Ni Y., Grossmann R., Zhao R. (2014). Comparative proteomic analysis of the breast muscle response to chronic corticosterone administration in broiler chickens showing long or short tonic immobility. *Poultry Science 93,* 784-793.

Gallup G.G.Jr., Boren J.L., Suarez S.D., Wallnau L.B., Gagliardi G.J. (1980). Evidence for the integrity of central processing during tonic immobility. *Physiology & Behavior 25 (2)*, 189-194.

Gallup G.G.Jr. (1977). Tonic immobility: The role of fear and predation. *The Psychological Record 27 (Suppl 1)*, 41-61.

Godin A.C., Worm B. (2010). Keeping the lead: how to strengthen shark conservation and management policies in Canada. *Marine Policy 34*, 995-1001.

Gonzalez R.J., McDonald D.G. (1992). The relationship between oxygen consumption and ion loss in a freshwater fish. *Journal of Experimental Biology 163*, 317-332.

Hammerschlag N., Gallagher A.J., Lazarre D.M. (2011). A review of shark satellite tagging studies. *Journal of Experimental Marine Biology and Ecology 398,* 1-8.

Hart N.S., Collin S.P. (2015). Sharks senses and shark repellents. *Integrative Zoology 10(1)*, 38-64.

Hawkins L.A., Armstrong J.D., Magurran A.E. (2004). Predator-induced hyperventilation in wild and hatchery Atlantic salmon fry. *Journal of Fish Biology 65*, 88-100.

Heithaus M.R. (2001). The biology of tiger sharks, *Galeocerdo cuvier*, in Shark Bay, Western Australia: sex ratio, size distribution, diet, and seasonal changes in catch rates. *Environmental Biology of Fishes 61*, 25-36.

Hennig C.W., Dunlap W.P. (1976). The effect of distance between predator and prey and the opportunity to escape on tonic mobility in *Anolis carolinensis*. *The Psychological Record 26*, 313-320.

Henningsen A.D. (1994). Tonic immobility in 12 elasmobranchs: use as an aid in captive husbandry. *Zoo Biology 13 (4)*, 325-332.

Hodgson E.S., Mathewson R.F. (1978). Electrophysiological studies of chemoreception in elasmobranchs. In: Hodgson E.S., Mathewson R.F. (editors). *Sensory Biology of Sharks, Skates, and Rays.* U.S. Government Printing Office, Washington, D.C., p. 227-267.

Hoffmayer E.R., Parsons G.R. (2001). The physiological response to capture and handling stress in the Atlantic sharpnose shark, *Rhizoprionodon terraenovae*. *Fish Physiology and Biochemistry 25*, 277-285.

Holland K.N., Wetherbee B.M., Lowe C.G., Meyer C.G., (1999). Movements of tiger sharks (*Galeocerdo cuvier*) in coastal Hawaiian waters. *Marine Biology 134*, 665–673.

Hughes J. (2009). The whale that ate jaws. Nature untamed. *National Geographic Channel 2*. https://blog.nationalgeographic.org/2011/11/25/nat-geo-wild-the-whale-that-ate-jaws/

Jorgensen S.J., Anderson S., Ferretti F., Tietz J.R., Chapple T., Kanive P., Bradley R.W., Moxley J.H., Block B.A. (2019). Killer whales redistribute white shark foraging pressure on seals. *Scientific Reports 9 (1)*, 6153.

Kempster R.M., McCarthy I.D., Collin S.P. (2012). Phylogenetic and ecological factors influencing the number and distribution of electroreceptors in elasmobranchs. *Journal of Fish Biology 80*, 2055-2088.

Kessel S.T., Hussey N.E. (2015). Tonic immobility as an anaesthetic for elasmobranchs during surgical implantation procedures. *Canadian Journal of Fisheries and Aquaculture Science 72,* 1-5.

Klemm W.R. (1971). Evoked responses in brain motor areas during the immobility reflex ("animal hypnosis"). *Physiology & Behavior 6(2)*, 137-144.

Klemm W.R. (1976). Identity of sensory and motor systems that are critical to the immobility reflex ("animal hypnosis"). *Journal of Neuroscience Research 2(1)*, 57-69.

Klimley P.A. (2013). Body design and swimming modes. In: Klimley P.A., Oerding S. (editors). *The Biology of Sharks and Rays.* The University of Chicago Press, Chicago USA, p. 51-82.

Kunze K., Simmons L. (2004). Notes on reproduction of the zebra shark, *Stegostoma fasciatum*, in a captive environment. In: Smith M., Warmolts D., Thoney D., Hueter R.E. (editors). *The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives.* Ohio Biological Survey, Columbus, p. 493-497.

Leach J. (2004). Why people 'freeze' in an emergency: temporal and cognitive constraints on survival responses. *Aviation Space and Environmental Medicine 75*, 539-542

Mandelman J.W., Skomal G.B. (2008). Differential sensitivity to capture stress assessed by blood acid–base status in five carcharhinid sharks. *Journal of Comparative Physiology B 179,* 267-277.

Manire C., Hueter R., Hull E., Spieler R. (2001). Serological changes associated with gill-net capture and restraint in three species of sharks. *Transactions of the American Fisheries Society 130,* 1038-1048.

Marx B.P., Forsyth J.P., Gallup G.G., Fusé T., Lexington J.M. (2008). Tonic immobility as an evolved predator defence: implications for sexual assault survivors. *Clinical Psychology: Science and Practice 15,* 74-90.

Möller A., Söndergaard H.P., Helström L. (2017). Tonic immobility during sexual assault – a common reactionpredicting post-traumatic stress disorder and severe depression. *Acta Obstetricia et Gynecologica Scandinavica 96,* 932-938.

Murchie K.J., Schwager E., Cooke S.J., Danylchuk A.J., Danylchuk E.E., Goldberg T.L.,

Suski C.D., Philipp D.P. (2009). Spatial ecology of juvenile lemon sharks (*Negaprion brevirostris*) in tidal creeks and coastal waters of Eleuthera, the Bahamas. *Environmental Biology of Fishes 89*, 95-104.

Otway N.M. (2015). Serum biochemical reference intervals for free living sand tiger sharks (*Carcharias taurus*) from east Australian waters. *Veterinary Clinical Pathology 44,* 262-274.

Petitjean Q., Jean S., Gandar A., Côte J., Laffaille P., Jacquin L. (2019). Stress responses in fish: From molecular to evolutionary processes. *Science of the Total Environment 684*, 371-380.

Powell R.A., Proulx G. (2003). Trapping and marking terrestrial mammals for research: integrating ethics, performance criteria, techniques, and common sense. *Institute of Laboratory Animal Resources Journal 44,* 259-276.

Pratt H.L., Carrier J.C. (2001). A review of elasmobranch reproductive behavior with a case study on the nurse shark, *Ginglymostoma cirratum*. *Environmental Biology of Fishes 60,* 157-188.

Primor N., Zlotkin E. (1975). On the ichthyotoxic and hemolytic action of the skin secretion of the flatfish *Pardachirus marmoratus* (Soleidae). *Toxicon 13(4),* 227-231.

Pyle P., Schramm M.J., Keiper C., Anderson S.D. (1999). Predation on a white shark (*Carcharodon carcharias*) by a killer whale (*Orcinus orca*) and a possible case of competitive displacement. *Marine Mammal Science 15(2)*, 563-568.

Randall D. (1982). The control of respiration and circulation in fish during exercise and hypoxia. *Journal of Experimental Biology 100*, 275-288.

Rasmussen L.E.L., Gruber S.H. (1993). Serum concentrations of reproductively-related circulating steroid hormones in the free-ranging lemon shark, *Negaprion brevirostris. Environmental Biology of Fishes 38(1)*, 167-174.

Rasmussen L.E.L., Schmidt M.J. (1992). Are sharks chemically aware of crocodiles? In: Doty R.L., Müller-Schwarze D. (editors). *Chemical Signals in Vertebrates 6.* Plenum Press, New York, p. 335-342.

Ratner S.C. (1967). Comparative aspects of hypnosis. In: Gordon J.E. (editor). *Handbook of Clinical and Experi-*

*mental Hypnosis*. Macmillan Publishing, New York, p. 550-587.

Richardson E.J., Shumaker M.J., Harvey E.R. (1977). The effects of stimulus presentation during cataleptic, restrained and free-swimming states on avoidance conditioning of goldfish (*Carassius auratus*). *The Psychological Record 27,* 63-75.

Roberts J.L. (1978). Active branchial and ram gill ventilation in fishes. *Biological Bulletin 148*, 85-105.

Romero L.M. (2004). Physiological stress in ecology: lessons from biomedical research. *Trends in Ecology and Evolution 19*, 249-255.

Schluessel V. Bennett M.B., Bleckmann H., Blomberg S., Collin S.P. (2008). Morphometric and ultrastructural comparison of the olfactory system in elasmobranchs: the significance of structure-function relationships based on phylogeny and ecology. *Journal of Morphology 269*, 1365-1386.

Shuttleworth T.J. (1988). Salt and water balance - extra renal mechanisms. In: Shuttleworth T.J. (editor). *Physiology of Elasmobranch Fishes*. Springer-Verlag, New York, p. 171-194.

Skomal G.B. (2007). Evaluating the physiological and physical consequences of capture on post-release survivorship in large pelagic fishes. *Fisheries Management and Ecology 14*, 81-89.

Skomal G.B., Bernal D. (2010). Physiological responses to stress in sharks. In: Carrier J.C., Musick J.A., Heithaus M.R. (editors). *Sharks and their Relatives II—Biodiversity, Adaptive Physiology and Conservation*. CRC Press Taylor Francis Group, Boca Raton, p. 457-488.

Springer S. (1955). Laboratory experiments with shark repellents. In: *Proceedings of the Seventh Annual Gulf and Caribbean Fisheries Institute*. Coral Gables, University of Miami, Institute of Marine Science, Florida. p. 159-164.

Stroud E.M., O'Connell C.P., Rice P.H., Snow N.H., Barnes B.B., Elshaer M.R., Hanson J.E. (2014). Chemical shark repellent: Myth or fact? The effect of a shark necromone on shark feeding behavior. *Ocean and Coastal Management 97*, 50-57.

Tachibana K., Gruber S.H. (1988). Shark repellent lipophilic constituents in the defence secretion of the Moses sole (*Pardachirus marmoratus*). *Toxicon 26 (9),* 839-853.

Tachibana K., Sakaitanai M., Nakanishi K. (1984). Pavoninins: shark-repelling ichthyotoxins from the defense secretion of the Pacific sole. *Science 226 (4675),* 703-705.

Tachibana K., Sakaitani M., Nakanishi K. (1985). Pavoninins, shark-repelling and ichthyotoxic steroid n-acetylglucosaminides from the defense secretion of the sole *Pardachirus pavoninus* (Soleidae). *Tetrahedron 41 (6),* 1027-1037.

Thompson R.K.R., Foltin R.W., Boylan R.J., Sweet A., Graves C.A., Lowitz C.E. (1981). Tonic immobility in Japanese quail can reduce the probability of sustained attack by cats. *Animal Learning and Behavior 9*, 145-149.

Voegeli F.A., Smale M.J., Webber D.M., Andrade Y., O'Dor R.K. (2001). Ultrasonic telemetry, tracking and automated monitoring technology for sharks. *Environmental Biology of Fishes 60,* 267-281.

Volchan E., Souze G.G., Franklin C.M., Norte C.E., Rocha-Rego V., Oliveira J.M., David I.A., Mendlowicz M.V., Coutinho E.S.F., Fiszman A., Berger W., Marques-Portella C., Figueira I. (2011). Is there tonic immobility in humans? Biological evidence from victims of traumatic stress. *Biological Psychology 88 (1),* 13-19.

Watsky M.A., Gruber S.H. (1990). Induction and duration of tonic immobility in the lemon shark, *Negaprion brevirostris*. *Fish Physiology & Biochemistry 8 (3)*, 207-210.

Weigmann S. (2016). Annotated checklist of the living sharks, batoids and chimaeras (Chondrichthyes) of the world, with a focus on biogeographical diversity. *Journal of Fish Biology 88 (3),* 837-1037.

Wells R.M.G., McNeil H., MacDonald J.A. (2005). Fish hypnosis: induction of an atonic immobility reflex. *Marine Behaviour and Physiology 38,* 71-78.

Whitman P.A., Marshall J.A., Keller E.C. Jr. (1986). Tonic immobility in the smooth dogfish shark, *Mustelus canis* (Pisces, Carcharhinidae). *Copeia 1986 (3)*, 829-832.

Williamson M.J., Dudgeon C., Slade R. (2018). Tonic immobility in the zebra shark, *Stegostoma fasciatum*, and its use for capture methodology. *Environmental Biology of Fishes 101(5)*, 741-748.

Williamson M.J., Kavanagh A.S., Noad M.J., Kniest E., Dunlop R.A. (2016). The effect of close approaches for tagging activities by small research vessels on the behavior of humpback whales (*Megaptera novaeangliae*). *Marine Mammal Science 32*, 1234-1253.

Woodruff M.L., Hatton D.C., Frankl M.B., Meyer, M.E. (1976). Effects of scopolamine and physostigmine on tonic immobility in ducks and guinea pigs. *Physiological Psychology 4(2),* 198-200.

Yao M., Rosenfeld J., Attridge S., Sidhu S., Aksenov V., Rollo C.D. (2009). The ancient chemistry of avoiding risks of predation and disease. *Evolutionary Biology 36 (3),* 267-281.

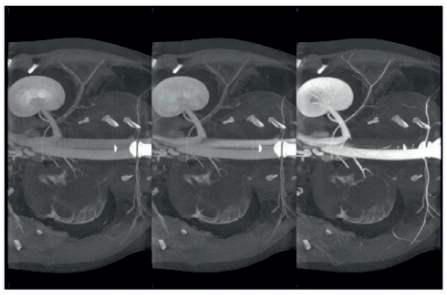
Zlotkin E., Gruber S.H. (1984). Synthetic surfactants: a new approach to the development of shark repellents. *Archives of Toxicology 56 (1)*, 55-58.

# X-Ray VERACHTERT Digital nv

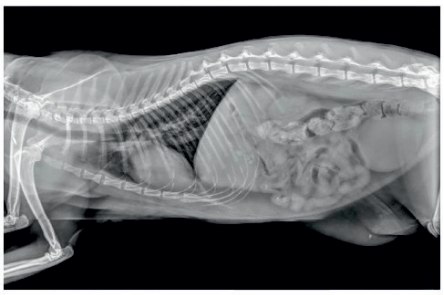
*Specialist medische beeldvorming sinds 1979 - RX - CR - DR - CT*

Koop bewust Belgisch! Wij zijn 100% Belgisch en zijn daar fier op.

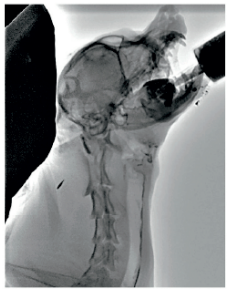
ANIMAGE



Cine-CT contrast scan



Lateral DR | overview filter

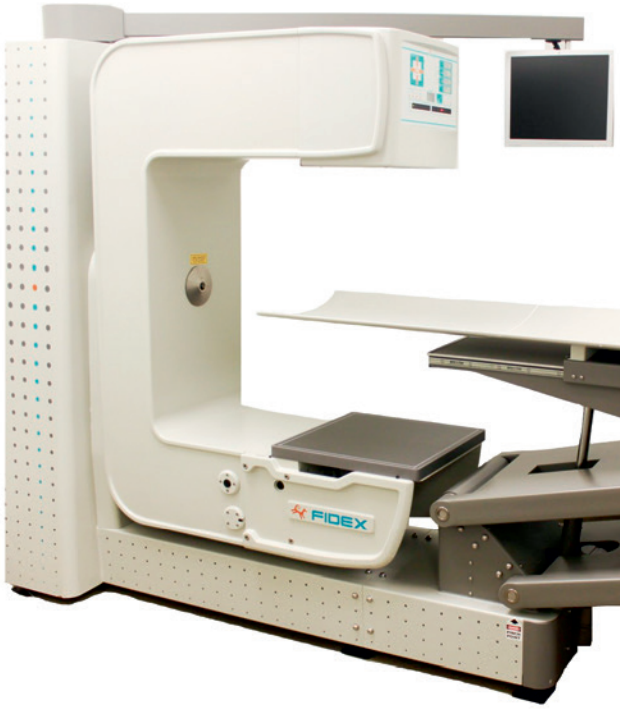


Watch video

FL | swallow study

MUSICA

AGFA HealthCare


FIDEX 3-in-1 Imaging Solutions for Veterinarians


new

Wi-Fi ONBOARD

**X-Ray Verachtert Digital nv**
Bisschoppenhoflaan 662
2100 Deurne - Belgium

phone :**+32 (0)3 239 05 79**
fax :**+32 (0)3 218 50 61**

mail : info@xrayverachtert.be
web : www.xrayverachtert.be

# Genetic insights in canine degenerative myelopathy

*Genetische inzichten in degeneratieve myelopathie bij honden*

**[1]F. Boeykens, [1]L. Peelman, [2,3]S. Bhatti, [1,3]B. J. G. Broeckx**

[1]Department of Nutrition, Genetics and Ethology, Faculty of Veterinary Medicine, Ghent University, Heidestraat 19, B-9820 Merelbeke, Belgium
[2]Small Animal Department, Small Animal Teaching Hospital, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium
[3]Both authors contributed equally

Bart.Broeckx@ugent.be

## ABSTRACT

**Canine degenerative myelopathy (DM) is a late-onset, progressive, neurodegenerative disorder with a fatal outcome, occurring in a vast number of dog breeds. Most dogs are at least eight years of age when they begin to show clinical signs, starting with general proprioceptive ataxia in the hind limbs and upper motor neuron paraparesis, evolving to lower motor neuron tetraplegia and brain stem signs. A definitive diagnosis can only be made postmortem by the histopathological observation of neuronal degradation and demyelination of the spinal cord. Most DM-affected dogs are homozygous for one of the known superoxide dismutase 1 gene (*SOD1*) mutations (ENSCAFT00000065394.1:c.82G>A, first described as NM_001003035.1:c.118G>A). A second mutation (NM_001003035.1:c.52A>T) in the same gene has been found but occurs only in Bernese mountain dogs. Not every homozygous dog develops the disease; this indicates that the disease is incompletely penetrant and that modifier loci might be present. In this review, the authors aim to give an overview of the disease progression and the current genetic knowledge of DM, which is of paramount importance for the correct diagnosis and to help reduce the disease incidence.**

## SAMENVATTING

Degeneratieve myelopathie bij honden is een progressieve neurodegeneratieve aandoening met een fatale afloop die voorkomt bij een groot aantal rassen. De meeste honden zijn minstens acht jaar oud wanneer ze klinische symptomen beginnen te vertonen, startend met paraparese en proprioceptieve ataxie in de achterpoten als gevolg van aantasting van de bovenste motorneuronen, waarbij de spinale reflexen nog intact zijn. Dit evolueert naar tetraplegie met verzwakte/afwezige spinale reflexen en het ontstaan van symptomen als gevolg van aantasting van de hersenstam. Een definitieve diagnose kan slechts post mortem worden gesteld door vaststelling van neuronale degradatie en demyelinisatie van het ruggenmerg via histopathologie. De meeste getroffen honden zijn homozygoot voor een van de gekende mutaties (ENSCAFT00000065394.1:c.82G>A, eerder beschreven als NM_001003035.1:c.118G>A) in het superoxide dismutase 1 gen (*SOD1*). Een tweede mutatie is bekend (NM_001003035.1:c.52A>T), maar komt enkel voor bij Berner sennenhonden. Aangezien niet elke homozygote hond de ziekte ontwikkelt, betekent dit dat de ziekte onvolledig penetrant is en dat er bovendien eventueel ziekte-modificerende loci aanwezig zijn. In dit artikel wordt beoogd een overzicht te geven van de ziekteprogressie en de huidige genetische kennis van degeneratieve myelopathie aangezien dit het startpunt is voor een correcte diagnose van de aandoening en het verminderen van de incidentie.

## INTRODUCTION

Canine degenerative myelopathy (DM) is a late-onset, neurodegenerative disorder with a fatal outcome, occurring in a vast number of dog breeds, with no sex predilection. Whereas the overall prevalence of DM, solely based on clinical signs and characteristics, in dogs is 0.19% (Coates et al., 2007; Wininger et al., 2011), these numbers are strongly breed-dependent. As such, this disease is far more common in dogs than in its human counterpart, amyotrophic lateral sclerosis (ALS), that occurs only in four to six individuals per 100,000, of which 10% of patients have a familial history (Tao and Wu, 2017). While a genetic diagnosis can be made as soon as a DNA sample can be obtained, which is basically at birth, the mean age-of-onset of clinical symptoms and diagnosis is nine years for larger dog breeds (Kathmann et al., 2006). However, among genetically affected dogs, the age-of-onset of clinical symptoms is highly variable and, as recently discovered, at least partially influenced by genetic modifiers (Ivansson et al., 2016). Because DM is notoriously difficult to diagnose, the progression of the disease and the normal clinical work-up will be discussed first. This is followed by an overview of the current genetic and molecular understanding of this disease and practical breeding advice.

## DISEASE PROGRESSION

Similar to ALS in humans, DM is characterized by both upper (UMN) and lower motor neuron (LMN) atrophy and death (Boillée et al., 2006). Whereas UMNs extend from the cerebral cortex or brainstem and carry information down to the spinal cord, LMNs connect the spinal cord with the skeletal muscles and are responsible for movement. Degeneration of the UMN results in modest weakness and spasticity, whereas LMN degeneration triggers more disabling weakness. When LMNs degenerate, the skeletal muscles no longer obtain the impulses necessary for movement and therefore begin to atrophy as well (Kato et al., 2008). The UMNs are first affected, followed afterwards by the LMNs; this also explains the evolution of symptoms observed during the disease progression.

Asymmetric UMN paraparesis, pelvic limb general proprioceptive ataxia with a T3-L3 neuroanatomic localization and lack of spinal hyperesthesia are the initial signs and key clinical features of DM (Olsonet al., 1982; Kathmann et al., 2006; Coates and Wininger, 2010).

At this stage, spinal reflexes generally remain intact (Griffiths and Duncan, 1975; Coates and Wininger, 2010). Often, dogs progress to non-ambulatory paraparesis and are euthanized during this disease stage. If the dog is not euthanized, the initial signs will progress to LMN paraplegia and ascend to affect the thoracic limbs. Urinary and fecal incontinence usually only develop in the later disease stages when paraplegia is present (Table 1). LMN signs emerge as hyporeflexia of the patellar and withdrawal reflexes, flaccid paralysis, and widespread muscle atrophy beginning at the pelvic limbs as the dogs become non-ambulatory. Flaccid tetraplegia occurs in dogs with advanced disease. Brainstem signs include swallowing difficulties and the inability to bark (Kathmann et al., 2006; Coates and Wininger, 2010).

## DIAGNOSING DM CLINICALLY

The diagnosis of DM is challenging, because the clinical signs in older dogs can be mimicked by several other neurologic and orthopedic diseases, such as degenerative lumbosacral syndrome, intervertebral disc disease, spinal cord neoplasia and degenerative joint diseases, e.g. hip dysplasia (Olson et al., 1982). As such, unfortunately, a firm diagnosis of DM can only be made postmortem by a histopathological examination of the spinal cord. Diagnosis premortem is mainly based on the exclusion of other neurological and/or orthopedic disorders with similar features, on the progressive and characteristic deterioration of the patient and on a positive genetic test (Table 1).

Although DM is most common in the German Shepherd Dog (GSD), it is also frequently reported in the Pembroke Welsh Corgi (PWC), Boxer, Rhodesian Ridgeback and Chesapeake Bay Retriever (Table 2). There is no sex predilection. The age-of-onset of clinical signs varies, but the mean age is nine years for larger dog breeds (Table 2). The time between the disease onset and end-stage can take up to three years with a mean of six months for larger dog breeds (Coates et al., 2007).

At an early stage, a presumptive diagnosis is based on the exclusion of other diseases. Firstly, paw replacement tests for proprioceptive positioning are informative as these tests show neurological deficits and exclude orthopedic conditions from the differential diagnosis. Next, imaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI) are used to rule out other spinal cord diseases (Kellett and Crocker, 2015). The imaging techniques are mainly used to exclude compressive spinal cord myelopathy. If MRI is unavailable, CT can also be performed. MRI is outstanding for imaging lesions of the brain, spinal cord and intervertebral discs, with a better soft tissue differentiation compared to CT.

Usually, in diagnosing DM, cerebrospinal fluid (CSF) analysis is done to rule out meningitis/myelitis as this should show a normal amount of total nucleated cell count and no protein abnormalities in DM-affected dogs. In the early stages of the disease, as in normal animals, no spontaneous muscle activity can be measured by electromyography (EMG) and axonal conduction rates remain normal. When dogs develop LMN signs, multifocal spontaneous muscle activity will appear on EMG and conduction studies will be

**Table 1. Overview of the sequence of clinical signs and diagnostic abnormalities encountered during disease progression. Degenerative myelopathy (DM) usually starts with upper motor neuron (UMN) pelvic limb (PL) paresis and general proprioceptive (GP) ataxia. When the disease progresses, lower motor neuron (LMN) weakness occurs and eventually also the thoracic limbs (TL) are affected. CSF: cerebrospinal fluid; EMG: electromyogram. Table adapted from Coates and Wininger (2010).**

| UMN Paraparesis and GP ataxia | LMN Paraparesis to Paraplegia | LMN Paraplegia to TL Weakness | LMN Tetraplegia and Brain Stem Signs |
|---|---|---|---|
| • Progressive general proprioceptive ataxia<br>• Asymmetric and spastic paraparesis<br>• Postural reaction deficits in PL<br>• Intact spinal reflexes (Patellar reflex may be decreased)<br>• Lack of paraspinal hyperesthesia | • Mild to moderate loss of muscle mass in PL<br>• Reduced to absent spinal reflexes in PL<br>• Nonambulatory paraparesis to paraplegia<br>• Potential urinary and fecal incontinence | • Signs of TL weakness<br>• Flaccid paraplegia<br>• Absence of spinal reflexes in PL<br>• Severe loss of muscle mass in PL<br>• Urinary and fecal incontinence | • Flaccid tetraplegia<br>• Difficulty with swallowing and tongue movements<br>• Absence of spinal reflexes in all limbs<br>• Reduced to absent cutaneous trunci reflex<br>• Generalized and severe loss of muscle mass<br>• Urinary and fecal incontinence |
| **Diagnostics – EARLY**<br>• Normal neuroimaging<br>• Normal electrodiagnostic testing<br>• Normal CSF analysis<br>• Homozygosity for SOD1:c.82G>A or SOD1:c.52A>T | | **Diagnostics – LATER**<br>• EMG abnormalities<br>• Nerve conduction studies show temporal dispersion and slow velocities | |
| **Disease onset** | 6-12 months | 9-18 months | 14-24 months | **End stage (> 36 months)** |

**Table 2. Common breeds affected by degenerative myelopathy (DM), mean age-of-onset (in years), mean disease duration (in months) and mean age of death (in years) for every breed. Values obtained from Coates and Wininger (2010).**

| Breed | Age-of-onset (μ) | Disease duration (μ) | Age of death (μ) |
|---|---|---|---|
| German Shepherd Dog | 8.6 | 15.8 | 9.8 |
| Pembroke Welsh Corgi | 10.9 | 20.0 | 12.6 |
| Chesapeake Bay Retriever | 9.1 | 17.8 | 10.6 |
| Boxer | 9.3 | 11.2 | 10.3 |
| Rhodesian Ridgeback | 7.8 | 7.7 | 8.0 |

μ: mean

abnormal (Awano et al., 2009). Finally, genetic tests can also be performed to confirm whether the patient is at least genetically at risk for DM.

Aside from these routinely used diagnostic techniques, new molecular techniques are being developed. In a recent study on PWCs, a positive correlation between increases in the plasma levels of miR-26b and disease progression has been discovered (Nakata et al., 2019). MiR-26b is a microRNA (miRNA), i.e. a small non-protein coding RNA. The plasma miR-26b has been suggested to be a novel diagnostic biomarker for DM and the combination with clinical examination is anticipated to enhance premortem diagnostics.

## HISTOPATHOLOGY

As stated before, a definitive diagnosis of DM is based on the postmortem histopathological examination of the spinal cord. Pathognomonic lesions are axonal and myelin degeneration at any level of the spinal cord. The degeneration is however generally more pronounced in thoracic regions and also proportional with the severity of clinical signs, whereas cervical and lumbar regions show more modest degeneration (March et al., 2009) (Figure 1). Funiculi, which are small bundles of axons enclosed by perineurium located within the white matter of spinal cord, are all

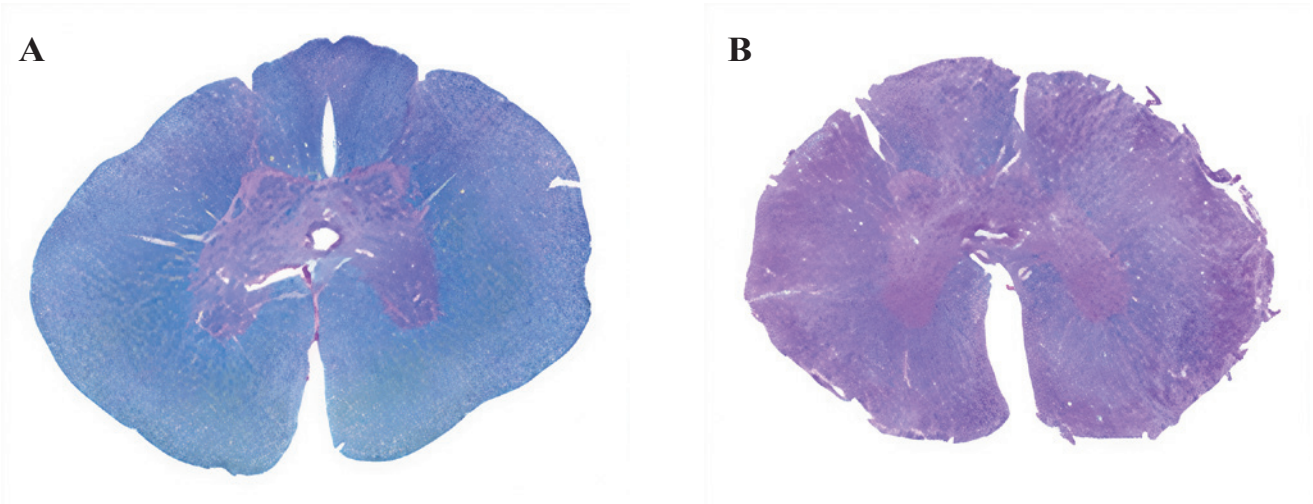



**Figure 1. Histopathological examination of the spinal cord. Luxol fast blue staining of a transversal section of a thoracic spinal cord segment of A. an unaffected dog and B. a dog affected by degenerative myelopathy (DM). The spinal cord of the DM-affected dog demonstrates more severe degeneration in the lateral and dorsal funiculi than the spinal cord of the unaffected dog. White matter degeneration is portrayed by distinct regions of pallor and the loss of blue color. Figure adapted from Kobatake et al. (2017).**

affected, but lesions are predominantly present in the dorsal fraction of the lateral funiculus (Griffiths and Duncan, 1975; Johnston et al., 2000). Affected dogs display distinct forms of axon cylinder vacuolization and loss, which is followed by astrogliosis (Johnston et al., 2001). While present in numerous breeds, DM has only been histopathologically confirmed in the GSD (Johnston et al., 2000), PWC (March et al., 2009), Boxer (Miller et al., 2009), Rhodesian Ridgeback (Awano et al., 2009), Chesapeake Bay Retriever, Siberian Husky (Bichsel et al., 1983) and the Miniature Poodle (Coates and Wininger, 2010), and breed-specific differences have been observed (Table 2). Affected GSDs demonstrate discontinuous, bilateral and asymmetric lesions. Findings on PWC were similar, but more severe and more widely spread, which might be a histopathological feature specific for small dog breeds (March et al., 2009). Knowledge of the effects of DM on brain tissue remains limited. In some studies, abnormalities have been found in certain parts of the brain using immunocytochemistry and electron microscopy, whereas in other studies, in which light microscopy was performed, abnormalities were not detected (Johnston et al., 2000; March et al., 2009).

## GENETIC ASPECTS OF DM

Due to the consistency of clinical signs and disease progression and the occurrence in certain but not all breeds, DM has been expected to have a genetic origin. The observation of familial segregation of DM in the PWC (Coates et al., 2007), the Siberian Husky and the Chesapeake Bay Retriever (Long et al., 2008) provided additional support for this hypothesis. Finally, in 2009, in a genome-wide association study, the first single nucleotide polymorphism was identified in the superoxide dismutase 1 *(SOD1)* gene (ENSCAFT00000065394.1:c.82G>A, first described as NM_001003035.1:c.118G>A) (rs853026434) responsible for DM (Awano et al., 2009). As mutations within this gene give rise to 2-10% of the familial cases of ALS (Majoor-Krakauer et al., 2003), this gene is a likely candidate for DM as well.

SOD1 is a highly conserved protein and is the major scavenger of cytoplasmatic superoxide radicals ($O_2^-$) and, as such, is important in processes associated with oxidative stress (Borchelt et al., 1994). Release of reactive oxygen species (ROS) contributes to a large extent to cell damage and death both through direct and indirect (e.g. apoptosis) signaling (Li et al., 2019). The SOD1 protein reduces and controls the malignant effects of ROS.

When compared to unaffected wild type control dogs, the mutation results in a transition from a glutamic acid to a lysine amino acid at position 28 (ENSCAFP00000013012.4:p.E28K, first described as p.E40K) within the folded protein. Despite the missense mutation, the diseased phenotype does not seem to be a consequence of loss of normal protein function (Sau et al., 2007; Sahin et al., 2017). The E28K substitution rather leads to a misfolded protein with reduced net negative charge, which makes it more susceptible to form aggregates due to reduced repulsion between individual proteins (Sandelin et al., 2007). It is exactly that aggregate formation that might be responsible for the observed neurodegeneration (Bruijn et al., 1998). In fact, it has recently been demonstrated that motor neurons and other cells actively take up SOD1 protein aggregates by endocytosis (Benkler et al., 2018). Furthermore, these SOD1 aggregates are also transported to neighboring cells within the spinal cord and this protein transfer is assisted by oligoden-

drocytes (Thomas et al., 2017). However, the exact pathophysiological effects of the SOD1 aggregates still need to be elucidated.

In addition to the SOD1:c.82G>A variant resulting in a E28K amino acid substitution, which is widely distributed in the overall dog population, another *SOD1* variant has been found, but solely in Bernese Mountain Dogs (BMDs) (NM_001003035.1:c.52A>T) (Wininger et al., 2011). In a study with 912 BMDs, an allele frequency of the SOD1:c.52A>T variant of 3.5% was found, which was considerably lower than the 38% frequency of the SOD1:c.82G>A allele in this breed (Zeng et al., 2014). Both the c.82G>A and c.52A>T mutations cause disease in a homozygous state, consistent with an autosomal recessive mode of inheritance. However, within the BMD, compound heterozygotes have also been found and 4 out of 24 of the dogs in the study by Zeng et al. (2014) actually developed clinical signs of DM.

## THE COMPLEX RELATION BETWEEN PHENOTYPE AND GENOTYPE

As DM is a disorder with a late onset, there can be a long lag in time between genetic diagnosis and the first symptoms. In addition, the age-of-onset seems to vary between breeds (Table 1). Amongst PWCs carrying two copies of the risk allele, some dogs develop DM fairly early (< 8 years), whereas others never demonstrate any signs (> 11 years) (Ivansson et al., 2016). This indicates that DM has incomplete penetrance, with penetrance being defined as how likely an individual is to present a specific physical trait, taking into account their genetic profile. The term complete penetrance indicates that every dog with a specific variant in a state that should result in disease, will develop the disease. In contrast, incomplete penetrance denotes that some dogs at risk might actually never demonstrate any clinical signs (Awano et al., 2009). Reduced penetrance has an impact on how genetic profiles should be interpreted and challenges geneticists to predict the probability that an individual dog will develop clinical symptoms. The overall prevalence of DM, solely based on clinical signs and not a positive genetic test, in Pembroke Welsch Corgi dogs and Cardigan Welsh Corgi dogs is 1.51% and 0.58%, respectively (Coates et al., 2007). However, the exact percentage of dogs, which are genetically at risk, that will become clinically affected is not yet determined. To obtain this information, prospective studies should be performed, where individuals are included in the study before the development of clinical signs and are followed up for years to collect data. One of the main drawbacks of this kind of study are the high expenses and that it is very time consuming.

In contrast to the PWCs, almost every Boxer, homozygous for the *SOD1* risk allele, develops DM (Ivansson et al., 2016). These breed-specific differences suggest that modifier loci might exist that influence disease risk. The identification of these genetic loci is expected to assist in the understanding of the DM etiology and can also be of clinical use for the prediction of disease progression in patients. In a recent study by Ivansson et al. using PWCs, a first modifier locus was found within the SP110 nuclear body protein gene (*SP110*), which is located on the 25$^{th}$ chromosome (cfa25). In the analysis, both affected and unaffected PWCs homozygous for the *SOD1* variant, were used to find potential modifier loci. A 'risk haplotype', found within the *SP110* was present in 40% of affected and merely 4% of unaffected dogs (Ivansson et al., 2016). This risk haplotype was generally present as a single copy, which implies that one copy is sufficient to have an influence on disease risk.

SP110 is part of the SP100/SP140 protein family of nuclear body proteins. These proteins are predominantly expressed in immune cells (Bloch et al., 2000). The SP110 protein affects fundamental cellular processes, such as transcription, apoptosis, senescence and reaction to DNA damage or infection (Lallemand-Breitenbach et al., 2010) and might also play a role in immune responses (Roscioli et al., 2006). This strengthens the hypothesis that neuroinflammation is an important feature of ALS (Appel et al., 2010). Future research should focus on elucidating the exact role of the *SP110* gene in DM, whereas the discovery of novel (and potentially even breed-specific) modifier loci might further clarify the variable age-of-onset.

As previously stated, miR-26b has been found to be associated with disease progression and might be an interesting novel biomarker (Nakata et al., 2019). However, its exact role has not been elucidated yet. A pathway analysis proposed that miR-26b mediates transcription of the amyloid beta precursor protein; however, the biological role of the protein has not been defined yet. Both miR-26b and amyloid beta precursor protein are active, upstream of the SOD1 expression cascade and might indirectly mediate its expression (Nakata et al., 2019).

## GENETIC TESTING AND BREEDING ADVICE

Genetic testing can be used in the diagnostical work-up of patients suspected to have DM, but also as a screening tool to identify carriers and dogs at risk and to give breeding advice. Identifying carriers is important as they can pass on the allele to future generations, without developing clinical signs themselves. However, due to the late onset of disease, even dogs homozygous for the mutant allele, usually only develop symptoms after the breeding age. Fortunately, DNA tests are commercially available (Mellersh et al., 2012). It is however important to use the correct DNA-test, taking the breed into account. For every breed, the SOD1:c.82G>A should be genotyped as this mutation is widely spread in numerous breeds. In addition, solely for the BMD, the SOD1:c.52A>T should be analyzed as well. Overall, dogs homozy-

gous (or compound heterozygous) for the *SOD1* mutations, are at risk of developing DM.

In the context of diagnosis, it is crucial not to draw conclusions from clinical signs and DNA-testing alone, as concomitant diseases may be present. It is of paramount importance to rule out every other possible origin of the deterioration to avoid wrong diagnosis and as a consequence, choices concerning euthanasia. Furthermore, as DM is a disease with incomplete penetrance, carrying two copies of the variant allele does not necessarily result in developing the disease.

In the context of screening and breeding advice, two (at first sight conflicting) concepts have to be kept in mind. Firstly, as genetic diversity is dangerously low in several breeds, the general focus should be to improve this. Practically, this means excluding as little animals as possible from breeding. Secondly, the goal is to reduce the prevalence of (genetic) diseases, which is usually achieved by excluding animals from breeding.

Conventional breeding methods, responsible for creating the domestic dog as we know it, have resulted in a remarkable reduction of the genetic diversity (Lindblad-toh et al., 2005; Wijnrocx et al., 2016). This reduction is often due to strong selective breeding practices, such as inbreeding and the use of a popular sire (Calboli et al., 2008). Each dog is undoubtedly carrier of mutant alleles that have no significant effect on the carriers' health. This is exemplified by a recent study that showed that nearly two in five dogs are carrier of at least one copy of a 'diseased allele' when screened for 152 currently known genetic disease-associated variants (Donner et al., 2018). However, when present in homozygous conditions, dogs can become affected. Decreasing genetic diversity will result in a higher frequency of a mutant allele and thus, the amount of homozygotes in the population (Donner et al., 2018). For this reason, breeding programs should focus on the prevention of hereditary disorders by reducing the causative genetic burden but at the same time aim to enhance genetic diversity (Marsden et al., 2016). Luckily, this can be achieved for DM as it is an autosomal recessive disorder.

Due to the recessive nature, the frequency of the mutant allele, and as a consequence also the number of affected dogs, can be reduced in a reasonable timeframe. Rather than completely excluding every carrier or even affected dogs from the breeding program, specific genotypes should be combined in such a way that no diseased animals are born (Broeckx et al., 2013). In autosomal recessive conditions, carrier dogs should not be combined with another carrier or an affected dog (Figure 2). Both dogs homozygous and heterozygous for the mutant allele should only be combined with wild type 'healthy' dogs. This is made possible by the use of DNA-tests. There is however an important remark when it comes to using dogs homozygous for the mutant allele in breeding programs.

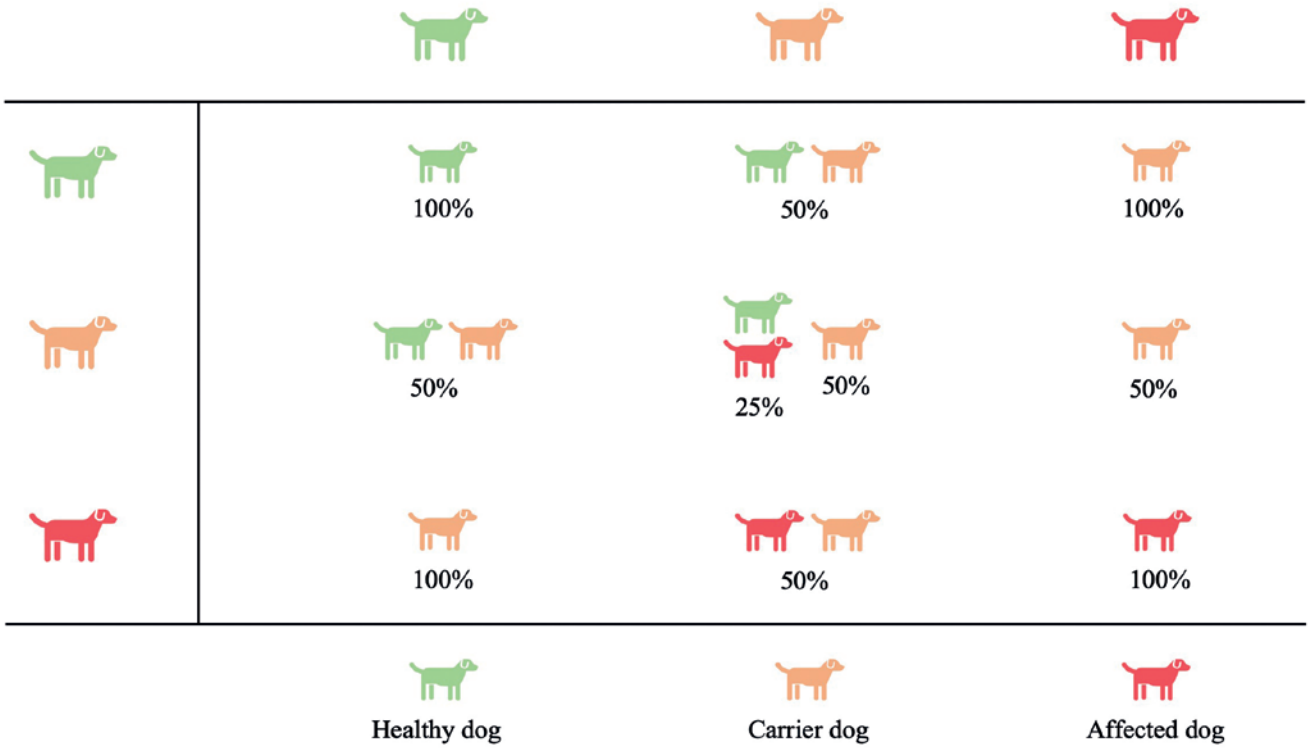



**Figure 2. The expected genotype frequencies and disease status in the progeny for all possible genotype combinations for phenotypes with an autosomal recessive mode of inheritance. Green dog: 'Healthy' dog for the disease of interest, carrying two copies of wild type allele; orange dog: 'Carrier' dog, having only one copy of the mutant allele; red dog: 'Affected' dog, homozygous for the disease variant, potentially displaying symptoms for the disease of interest.**

Dogs homozygous for the variant allele can be combined with healthy dogs, as offspring will be 100% carrier and thus, will not develop DM. However, it is of paramount importance to take the welfare of the animal into account. If the welfare has not been compromised at the time of breeding, there is in fact no problem in further use of the animal. If the animal is already starting to show symptoms and thus is unable to cope with pregnancy or breeding in general, it is strongly discouraged to continue using the animal for breeding purposes.

Next to carefully combining the correct genotypes for mating, it is important not to overuse a carrier or affected dog, as this might increase the frequency of the mutated allele in the population. This is however in line with the general recommendation to avoid the overuse of specific animals in breeding.

## CONCLUSION

Whereas the inheritance pattern of DM is simple, DM proves to be a phenotypically complex progressive disorder, similar to ALS in humans. It displays a characteristic pattern of clinical signs and is widespread in pedigree and mixed breed dogs. DM has a late onset, generally between eight and eleven years, and both sexes are equally affected.

The *SOD1* variant has been labeled as a major causative agent for developing DM. However, the inconsistent age-of-onset of clinical symptoms suggests that modifier loci might influence disease risk, as has recently been proven by the identification of the *SP110* risk haplotype. Detection and identification of these genetic loci are expected to assist in the interpretation and understanding of DM etiology. Due to the recessive autosomal nature of the *SOD1* mutation, the breeding advice is to carefully combine specific genotypes for breeding, rather than to completely exclude every carrier or even affected dogs from the breeding program as this would attribute to a further reduction of genetic diversity.

## REFERENCES


Appel, S. H., Beers, D. R., Henkel, J. S. (2010). T cell-microglial dialogue in Parkinson's disease and amyotrophic lateral sclerosis: are we listening? *Trends in Immunology 31*, 7-17. https://doi.org/10.1016/j.it.2009.09.003

Awano, T., Johnson, G. S., Wade, C. M., Katz, M. L., Johnson, G. C., Taylor, J. F., Perloski, M., Biagi, T., Baranowska, I., Long, S., March, P. A., Olby, N., Shelton, D., Khan, S., O'Brien, D. P., Lindblad-Toh, K., Coates, J. R. (2009). Genome-wide association analysis reveals a SOD1 mutation in canine degenerative myelopathy that resembles amyotrophic lateral sclerosis. In: *Proceedings of the National Academy of Sciences 106*, 2794-2799. https://doi.org/10.1073/pnas.0812297106

Benkler, C., O'Neil, A. L., Slepian, S., Qian, F., Weinreb, P. H., Rubin, L. L. (2018). Aggregated SOD1 causes selective death of cultured human motor neurons. *Scientific Reports 8*, 1-14. https://doi.org/10.1038/s41598-018-34759-z

Bichsel, P., Vandevelde, M., Lang, J., Kull-Hächler, S. (1983). Degenerative myelopathy in a family of Siberian Husky dogs. *Journal of the American Veterinary Medical Association 183*, 998-1000.

Bloch, D. B., Nakajima, A., Gulick, T., Chiche, J.-D., Orth, D., de la Monte, S. M., Bloch, K. D. (2000). Sp110 Localizes to the PML-Sp100 nuclear body and may function as a nuclear hormone receptor transcriptional coactivator. *Molecular and Cellular Biology 20*, 6138-6146. https://doi.org/10.1128/mcb.20.16.6138-6146.2000

Boillée, S., Vande Velde, C., Cleveland, D. W. W. (2006). ALS: A disease of motor neurons and their nonneuronal neighbors. *Neuron 52*, 39-59. https://doi.org/10.1016/j.neuron.2006.09.018

Borchelt, D. R., Lee, M. K., Slunt, H. S., Guarnieri, M., Xu, Z. S., Wong, P. C., Brown, R. H., Price, D. L., Sisodia, S. S., Cleveland, D. W. (1994). Superoxide dismutase 1 with mutations linked to familial amyotrophic lateral sclerosis possesses significant activity. In: *Proceedings of the National Academy of Sciences of the United States of America*, *91*, 8292-8296. https://doi.org/10.1073/pnas.91.17.8292

Broeckx, B. J. G., Coopman, F., Verhoeven, G. E. C., Van Haeringen, W., van de Goor, L., Bosmans, T., Gielen, I., Saunders, J. H., Soetaert, S. S. A., Van Bree, H., Van Neste, C., Van Nieuwerburgh, F., Van Ryssen, B., Verelst, E., Van Steendam, K., Deforce, D. (2013). The prevalence of nine genetic disorders in a dog population from Belgium, the Netherlands and Germany. *PLoS ONE*, *8*(9), 1-8. https://doi.org/10.1371/journal.pone.0074811

Bruijn, L. I., Houseweart, M. K., Kato, S., Anderson, K. L., Anderson, S. D., Ohama, E., Reaume, A. G., Scott, R. W., Cleveland, D. W. (1998). Aggregation and motor neuron toxicity of an ALS-linked SOD1 mutant independent from wild-type SOD1. *Science 281*, 1851-1854. https://doi.org/10.1126/science.281.5384.1851

Calboli, F. C. F., Sampson, J., Fretwell, N., Balding, D. J. (2008). Population structure and inbreeding from pedigree analysis of purebred dogs. *Genetics 179*, 593-601. https://doi.org/10.1534/genetics.107.084954

Coates, J. R., March, P. A., Oglesbee, M., Ruaux, C. G., Olby, N. J., Berghaus, R. D., O'Brien, D. P., Keating, J. H., Johnson, G. S., Williams, D. A. (2007). Clinical characterization of a familial degenerative myelopathy in Pembroke Welsh Corgi dogs. *Journal of Veterinary Internal Medicine 21*, 1323-1331. https://doi.org/10.1892/07-059.1

Coates, J. R., Wininger, F. A. (2010). Canine degenerative myelopathy. *Veterinary Clinics of North America - Small Animal Practice 40*, 929-950. https://doi.org/10.1016/j.cvsm.2010.05.001

Donner, J., Anderson, H., Davison, S., Hughes, A. M., Bouirmane, J., Lindqvist, J., Lytle, K. M., Ganesan, B., Ottka, C., Ruotanen, P., Kaukonen, M., Forman, O. P., Fretwell, N., Cole, C. A., Lohi, H. (2018). Frequency and distribution of 152 genetic disease variants in over 100,000 mixed breed and purebred dogs. *PLoS Genetics 14*, 1-20. https://doi.org/10.1371/journal.pgen.1007361

Griffiths, I. R., & Duncan, I. D. (1975). Chronic degenerative radiculomyelopathy in the dog. *Journal of Small Animal Practice 16*, 461-471. https://doi.org/10.1111/j.1748-5827.1975.tb05773.x

Ivansson, E. L., Megquier, K., Kozyrev, S. V., Murén, E., Körberg, I. B., Swofford, R., Koltookian, M., Tonomura, N., Zeng, R., Kolicheski, A. L., Hansen, L., Katz, M. L., Johnson, G. S., Coates, J. R., Lindblad-Toh, K. (2016). Variants within the SP110 nuclear body protein modify risk of canine degenerative myelopathy. In: *Proceedings of the National Academy of Sciences 113*, E3091-E3100. https://doi.org/10.1073/pnas.1600084113

Johnston, P. E. J., Barrie, J. A., McCulloch, M. C., Anderson, T. J., Griffiths, I. R. (2000). Central nervous system pathology in 25 dogs with chronic degenerative radiculomyelopathy. *The Veterinary Record 146*, 629-633. https://doi.org/10.1136/vr.146.22.629

Johnston, P. E. J., Knox, K., Gettinby, G., Griffiths, I. R. (2001). Serum α-tocopherol concentrations in German shepherd dogs with chronic degenerative radiculomyelopathy. *Veterinary Record 148*, 403-407. https://doi.org/10.1136/vr.148.13.403

Kathmann, I., Cizinauskas, S., Doherr, M. G., Steffen, F., Jaggy, A. (2006). Daily controlled physiotherapy increases survival time in dogs with suspected degenerative myelopathy. *Journal of Veterinary Internal Medicine 20*, 927-932. https://doi.org/10.1111/j.1939-1676.2006.tb01807.x

Kato, S. (2008). Amyotrophic lateral sclerosis models and human neuropathology: Similarities and differences. *Acta Neuropathologica 115*, 97-114. https://doi.org/10.1007/s00401-007-0308-4

Kellett, C. G., Crocker, M. J. (2015). Compressive myelopathy. *Surgery (United Kingdom) 33*, 390-399. https://doi.org/10.1016/j.mpsur.2015.05.004

Kobatake, Y., Sakai, H., Tsukui, T., Yamato, O., Kohyama, M., Sasaki, J., Kato, S., Urushitani, M., Maeda, S., Kamishina, H. (2017). Localization of a mutant SOD1 protein in E40K-heterozygous dogs: Implications for non-cell-autonomous pathogenesis of degenerative myelopathy. *Journal of the Neurological Sciences 372*, 369-378. https://doi.org/10.1016/j.jns.2016.10.034

Lallemand-Breitenbach, V., de Thé, H. (2010). PML nuclear bodies. *Cold Spring Harbor Perspectives in Biology 2*, 1-18. https://doi.org/10.1101/cshperspect.a000661

Li, X., Chen, Y., Zhao, J., Shi, J., Wang, M., Qiu, S., Hu, Y., Cui, Y., Liu, C. (2019). The specific inhibition of SOD1 selectively promotes apoptosis of cancer cells via regulation of the ROS signaling network. *Oxidative Medicine and Cellular Longevity* . https://doi.org/10.1155/2019/9706792

Lindblad-toh, K., Wade, C. M., Mikkelsen, T. S., Karlsson, E. K., Jaffe, D. B., Kamal, M., Clamp, M., Chang, J. L., Kulbokas III, E. J., Zody, M. C., Mauceli, E., Xie, X., Breen, M., Wayne, R. K., Ostrander, E. A., Ponting, C. P., Galibert, F., Smith, D. R., DeJong, P., Kirkness, J., Alvarez, P., Biagi, T., Brockman, W., Butler, J., Chin, C., Cook, A., Cuff, J., Daly, M. J., DeCaprio, D., Gnerre, S., Grabherr, M., Kellis, M., Kleber, M., Bardeleben, G., Goodstadt, L., Heger, A., Hitte, C., Kim, L., Koepfli, K., Parker, H., Pollinger, J. P., Searle, S. M. J., Sutter, N. B., Thomas, R., Webber, C., Broad Institute Genome Sequencing Platform, Lander, E. S. (2005). Genome. *Nature 438*, 803-819. https://doi.org/10.1038/nature04338

Long, S., Henthorn, P., Serpell, J., Van Winkle, T., Coates, J. (2008). Degenerative myelopathy in chesapeake bay retrievers. *Journal of Veterinary Internal Medicine 23*, 401-402. https://doi.org/10.1111/j.1939-1676.2009.00250.x

Majoor-Krakauer, D., Willems, P. J., Hofman, A. (2003). Genetic epidemiology of amyotrophic lateral sclerosis. *Clinical Genetics 63*, 83-101. https://doi.org/10.1046/j.0009-9163.2002.00001.x

March, P. A., Coates, J. R., Abyad, R. J., Williams, D. A., O'Brien, D. P., Olby, N. J., Keating, J. H., Oglesbee, M. (2009a). Degenerative myelopathy in 18 Pembroke Welsh Corgi dogs. *Veterinary Pathology 46*, 241-250. https://doi.org/10.1354/vp.46-2-241

Marsden, C. D., Vecchyo, D. O. Del, O'Brien, D. P., Taylor, J. F., Ramirez, O., Vilà, C., Marques-Bonet, T., Schnabel, R. D., Wayne, R. K., Lohmueller, K. E. (2016). Bottlenecks and selective sweeps during domestication have increased deleterious genetic variation in dogs. In: *Proceedings of the National Academy of Sciences of the United States of America 113*, 152-157. https://doi.org/10.1073/pnas.1512501113

Mellersh, C. (2012). DNA testing and domestic dogs. *Mammalian Genome 23*, 109-123. https://doi.org/10.1007/s00335-011-9365-z

Miller, A. D., Barber, R., Porter, B. F., Peters, R. M., Kent, M., Platt, S. R., Schatzberg, S. J. (2009). Brief communication: Degenerative myelopathy in two boxer dogs. *Veterinary Pathology 46*, 684-687. https://doi.org/10.1354/vp.08-VP-0270-M-BC

Nakata, K., Heishima, K., Sakai, H., Yamato, O., Furusawa, Y., Nishida, H., Sadatoshi, M., Kamishina, H. (2019). Plasma microRNA miR-26b as a potential diagnostic biomarker of degenerative myelopathy in Pembroke welsh corgis. *BMC Veterinary Research 15*, 1-9. https://doi.org/10.1186/s12917-019-1944-3

Olson, P., Carithers, R. W., Carithers, R. W. (1982). Differential diagnosis of conditions mimicking intervertebral disc disease in the canine. *Iowa State Veterinarian 44*, 60-65.

Roscioli, T., Cliffe, S. T., Bloch, D. B., Bell, C. G., Mullan, G., Taylor, P. J., Sarris, M., Wang, J., Donald, J. A., Kirk, E. P., Ziegler, J. B., Salzer, U., McDonald, G. B., Wong, M., Lindeman, R., Buckley, M. F. (2006). Mutations in the gene encoding the PML nuclear body protein Sp110 are associated with immunodeficiency and hepatic veno-occlusive disease. *Nature Genetics 38*, 620-622. https://doi.org/10.1038/ng1780

Sahin, A., Held, A., Bredvik, K., Major, P., Achilli, T.-M., Kerson, A. G., Wharton, K., Stilwell, G., Reenan, R. (2017). Human SOD1 ALS mutations in a droso phila knock-in model cause severe phenotypes and reveal dosage-sensitive gain and loss of function components. *Genetics 205*, 707-723. https://doi.org/10.1534/genetics.116.190850

Sandelin, E., Nordlund, A., Andersen, P. M., Marklund, S. S. L., Oliveberg, M. (2007). Amyotrophic lateral sclerosis-associated copper/zinc superoxide dismutase mutations preferentially reduce the repulsive charge of the proteins. *Journal of Biological Chemistry 282*, 21230-21236. https://doi.org/10.1074/jbc.M700765200

Sau, D., De Biasi, S., Vitellaro-Zuccarello, L., Riso, P., Guarnieri, S., Porrini, M., Simeoni, S., Crippa, V., Onesto, E., Palazzolo, I., Rusmini, P., Poletti, A. (2007). Mutation of SOD1 in ALS: A gain of a loss of function. *Human Molecular Genetics 16*, 1604-1618. https://doi.org/10.1093/hmg/ddm110

Tao, Q. Q., Wu, Z. Y. (2017). Amyotrophic lateral sclerosis: Precise diagnosis and individualized treatment. *Chinese Medical Journal 130*, 2269-2272. https://doi.org/10.4103/0366-6999.215323

Thomas, E. V., Fenton, W. A., McGrath, J., Horwich, A. L. (2017). Transfer of pathogenic and nonpathogenic cytosolic proteins between spinal cord motor neurons in vivo in chimeric mice. In: *Proceedings of the National Academy of Sciences of the United States of America 114*, E3139–E3148. https://doi.org/10.1073/pnas.1701465114

Wijnrocx, K., François, L., Stinckens, A., Janssens, S., Buys, N. (2016). Half of 23 Belgian dog breeds has a compromised genetic diversity, as revealed by genealogical and molecular data analysis. *Journal of Animal Breeding and Genetics*, 1-9. https://doi.org/10.1111/jbg.12203

Wininger, F. A., Zeng, R., Johnson, G. S., Katz, M. L., Johnson, G. C., Bush, W. W., Jarboe, J. M., Coates, J. R. (2011). Degenerative myelopathy in a Bernese mountain dog with a novel SOD1 missense mutation. *Journal of Veterinary Internal Medicine 25*, 1166-1170. https://doi.org/10.1111/j.1939-1676.2011.0760.x

Zeng, R., Coates, J. R., Johnson, G. C., Hansen, L., Awano, T., Kolicheski, A., Ivansson, E., Perloski, M., Lindblad-Toh, K., O'Brien, D. P., Guo, J., Katz, M. L., Johnson, G. S. (2014). Breed distribution of SOD1 alleles previously associated with canine degenerative myelopathy. *Journal of Veterinary Internal Medicine 28*, 515-521. https://doi.org/10.1111/jvim.12317

**Persbericht**

# Zoetis lanceert nieuwe selectietool CLARIFIDE Plus voor een gezondere en rendabelere veestapel

Management- en selectietool CLARIFIDE® Plus van Zoetis is nu ook beschikbaar voor de Belgische melkveehouderij. De service omvat het bepalen van genomische fokwaarden met een merkertest, de online selectie-applicatie SearchPoint en begeleiding door een specialist. Melkveehouders krijgen hiermee inzicht in het genetisch potentieel van hun veestapel. Op basis hiervan kunnen ze strategischer (jong)vee selecteren en gerichtere fokkerijbeslissingen nemen die diergezondheid verbeteren en het rendement verhogen. CLARIFIDE Plus is zowel via Zoetis als via samenwerkingspartner WWS te bestellen.

Bij CLARIFIDE® Plus wordt de genetische aanleg van een kalf (of koe) bepaald uit het DNA van een oorbiopt (voor een betrouwbare uitslag voert het dier minimaal 87,5% Holstein-bloed). Daarmee is het genetisch potentieel van een dier al op jonge leeftijd te bepalen. De test en de bijbehorende selectie-applicatie bieden de melkveehouder de mogelijkheid om samen met de bedrijfsdierenarts vooruit te kijken en betere selectiebeslissingen te nemen wat betreft het jongvee dat hij of zij opfokt. Ook kan de melkveehouder strategischer fokkerijkeuzes maken op basis van genetische eigenschappen die passen bij de ondernemer en de bedrijfsdoelen. Zo kunnen melkveehouders hun beschikbare productieruimte optimaal benutten en met hun fok- en selectiebeleid gericht sturen op diergezondheid en rendement.

CLARIFIDE Plus combineert gezondheid, vruchtbaarheid, productie en een functioneel exterieur in één onafhankelijke totaalindex die de economische waarde (rendement) uitdrukt: de Dairy Wellness Profit index. Dankzij genomische fokwaarden beschikt de melkveehouder over informatie die betrouwbaarder is dan wanneer alleen gekeken wordt naar de fokwaarden op basis van afstamming en eigen prestaties.
www.clarifideplus.be

CLARIFIDE® plus

# VOORSPELLEN, SELECTEREN EN MANAGEN MET DIERGEZONDHEIDS-KENMERKEN


- vroeg inzicht in genetisch potentieel vaarskalveren
- selecteren op koe- en kalfgezondheidskenmerken
- sturen op gezondere veestapel én hoger rendement

**WWW.CLARIFIDEPLUS.BE**



Zoetis Belgium SA
Mercuriusstraat 20
1930 Zaventem
www.zoetis.be
info.be@zoetis.com



MM-10285

zoetis

# Vaginal prolapse complicated with urinary bladder retroflexion and colonic herniation in a dog

*Vaginale verzakking gecompliceerd met retroflexie van de blaas en hernia van de dikke darm bij een hond*

**[1*]O. F. Yesilkaya, [1]M. F. Ciftci, [1]F. Satilmis, [2]K. Parlak, [1]H. Alkan, [1]H. Erdem**

[1]Selcuk University, Faculty of Veterinary Medicine, Departmant of Veterinary Obstetrics and Gynecology, Alaeddin Keykubat Campus, Konya, Turkey
[2]Selcuk University, Faculty of Veterinary Medicine, Departmant of Veterinary Surgery, Alaeddin Keykubat Campus, Konya, Turkey

faruk.yesilkaya@selcuk.edu.tr

## ABSTRACT

**A four-year-old, intact, female Anatolian Shepherd dog was presented with a three-day vaginal prolapse and anuria. She was lethargic, dehydrated, tachycardic, and blood analysis showed leukocytosis and azotemia. Ultrasonographic examination demonstrated that the urinary bladder was located in the prolapsed vaginal tissue. Ultrasound-guided cystocentesis was performed to empty the obstructed bladder and intravenous fluid therapy was instituted. When the dog was deemed cardiovascularly stable, a caudal midline celiotomy incision was made. Through gentle retraction of the uterus, the colon descendens and the bladder were placed back to their normal positions. After resolution of the cervical invagination, the cervix was pexied to the abdominal wall to prevent recurrence and ovariohysterectomy was performed. The dog made an uneventful recovery and had normal urination at the one month follow-up. Chronic vaginal prolapse can be complicated by a retroflexed urinary bladder with urethral obstruction leading to life-threatening azotemia. Ultrasonography of the prolapsed tissues contributes greatly to early diagnosis of complicated cases.**

## SAMENVATTING

Een vier jaar oude, intacte, vrouwelijke Anatolische herdershond werd aangeboden met een vaginale verzakking en anurie die reeds drie dagen aanwezig waren. De hond vertoonde lethargie, dehydratatie en tachycardie; bloedanalyse toonde leukocytose en azotemie aan. Op echografisch onderzoek werd vastgesteld dat de urineblaas zich in het verzakte vaginale weefsel bevond. Echogeleide cystocentese werd uitgevoerd om de verstopte blaas te ledigen en er werd intraveneuze vloeistoftherapie ingesteld. Toen de hond cardiovasculair stabiel werd bevonden, werd de buikwand geopend in de middenlijn via een incisie caudaal van de navel. Door voorzichtige retractie van de baarmoeder werden de colon descendens en de blaas teruggebracht naar hun normale posities. Nadat de cervicale invagatie was verdwenen, werd de cervix (door middel van hechtingen) aan de buikwand gefixeerd om recidief te voorkomen. Daarna werd een ovariohysterectomie uitgevoerd. De hond herstelde zonder problemen. Bij het controleonderzoek één maand na de ingreep urineerde ze normaal. Chronische vaginale verzakking kan worden bemoeilijkt door een retroflexe urineblaas met urethrale obstructie, wat tot levensbedreigende azotemie kan leiden. Echografische onderzoek van de verzakte weefsels draagt in hoge mate bij tot de vroege diagnose van gecompliceerde gevallen.

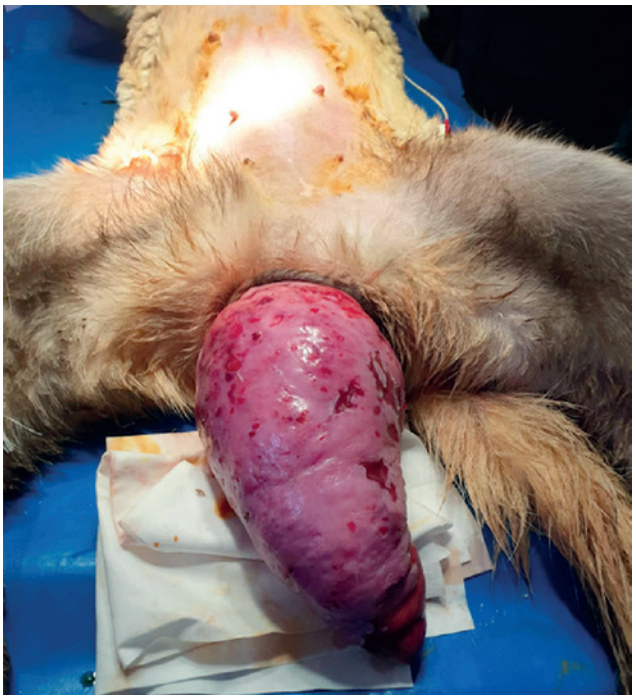


**Figure 1. Ventral view on the vaginal prolapse demonstrating the severely congested vaginal mucosa with superficial erosions.**

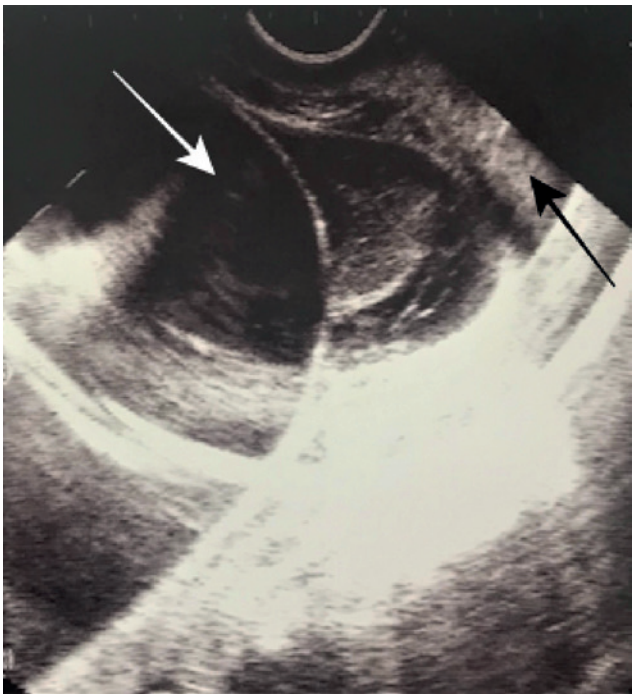


**Figure 2. Ultrasonographic view of the urinary bladder in the prolapsed vaginal tissue. White arrow: bladder, black arrow: vaginal tissue.**

## INTRODUCTION

Vaginal prolapse in dogs is recognized as the protrusion of donut-shaped edematous vaginal tissue from the vulva (Sontas et al., 2010). It is more common in young dogs (<3 years), large breed dogs (Boxer, Mastiff, and Anatolian Shepherd dogs), and dogs having their first estrus upon reaching puberty (Schaefers-Okkens, 2001; Nak and Kaşıkçı, 2013). It is more common in proestrus and early estrus periods of the reproductive cycle, during which estrogen hormone concentration is high (Sontas et al., 2010). The other causes of vaginal prolapse are considered to be constipation, dystocia or forced separation during mating. In pregnant dogs, the decrease in progesterone and increase in estrogen levels, and relaxin levels close to the parturation are important predisposing factors (Alan et al., 2007; Gouletsou et al., 2009). However, it can be rarely seen in diestrus in untreated chronic cases (Johnston et al., 2001). In addition, a case of vaginal prolapse has been reported as a side effect of exogenous estradiol benzoate administration used to induce estrus in a dog with prolonged anestrous (Sarrafzadeh-Rezaei et al., 2008).

When the vaginal prolapse goes unnoticed by the owners, or no treatment is given, further complications might occur (Feldman and Nelson, 2004; Alan et al., 2007). Female dogs with vaginal prolapse have no desire to mate; and even, when desire would be present, penetration can not occur during mating (Johnston et al., 2001). Decreased circulation in the prolapsed part of the vagina evolves from extensive edema and hemorrhage to necrosis (Feldman and Nelson, 2004; Sontas et al., 2010). This can be further exacerbated by automutilation. Also, pelvic organs might become entrapped in the vaginal prolapse leading to herniation into the prolapsed vaginal tissue (Ober et al., 2016). Herniation of the bladder as a complication of vaginal prolapse has been described (Alan et al., 2007; Canatan et al., 2015; Acar et al., 2017; Özgenç et al., 2017). The combination of vaginal and rectal prolapse has also been described (Ober et al., 2016). A potentially life-threatening complication is partial or total urethral occlusion leading to dysuria or anuria (Schaefers-Okkens, 2001; Sontas et al., 2010).

Retroflexion of the bladder in dogs is usually associated with degeneration of the pelvic diaphragm and perineal hernia (White et al., 1986). This is usually seen in male dogs and rarely in female dogs (Sontas et al., 2008; Adeyanju et al., 2011). In this case report, the treatment of a rare chronic vaginal prolapse complicated with urinary bladder retroflexion and colonic hernia in a dog is described.

## CASE REPORT

A four-year-old, intact, female Anatolian Shepherd dog of 43 kg was presented to the Selcuk University Veterinary Faculty Obstetrics and Gynecology Clinic. Approximately two to three weeks before presentation, a mass had appeared, protruding from the vulva. This mass gradually enlarged over time. The dog initially had symptoms limited to urinary straining, but later developed dysuria. The dog had become anuric three days prior to presentation and her general condition was deteriorating. Clinical examination revealed

weightloss, 6% dehydration (dry oral mucous membranes, eyes moist, mild loss of skin turgor), tachypnea, tachycardia, lethargia and a large, donut-shaped mass protruding from the vulva. Because of the central lumen, this was identified as a vaginal prolapse with edematous, hemorrhagic and superficially necrotic mucosa (Figure 1).

To evaluate the dog's general health, a complete blood count and biochemistry were performed revealing leukocytosis and azotemia (Table 1). Because of tachypnea and tachycardia, a blood gas analysis was also performed (Table 2). Despite the low bicarbonate level and base deficit in the blood, pH remained within the reference values due to the buffer and respiratory compensatory mechanism.

An attempt was made to place a urinary catheter, but this was not possible since the external urethral orifice could not be identified in the abnormal prolapsed tissue. Ultrasonographic examination showed that the urinary bladder was not located in the abdominal cavity; it was detected in the prolapsed vaginal tissues (Figure 2). Ultrasound-guided cystocentesis was performed to empty the obstructed bladder (Figure 3). Next, a bolus of lactated Ringer's solution was administered intravenously followed by a maintenance dose of 5 ml /kg/hr (PF Lactated Ringer Solution, Polifarma, Turkey). The dog also received oxygen supplementation through a mask.

As soon as the dog was considered cardiovascularly stable, she was scheduled for surgical treatment. As premedication, 20 µg/kg medetomidine (Domitor, Vetoquinol, UK) was administered intravenously ten minutes before induction. Induction of anesthesia was performed IV with 6 mg/kg propofol (Propofol 1%, Fresenius, Germany). Following endotracheal intubation of the dog, inhalation anesthesia was performed with 2% isoflurane (Isofurane® 100 ml, Adeka, Turkey) in an oxygen mixture. As a preoperative antibiotic, 7 mg/kg amoxicillin-clavulanic acid (Synulox RTU, Zoetis, USA) was given subcutaneously 2.5 hours before surgery.

The dog was positioned in dorsal recumbency with the vulva and vaginal prolapse in the surgical field. Following fluid therapy, cystocentesis was again performed to evaluate the effect of the fluid therapy and to empty the bladder before surgery. The abdominal and perineal regions were prepared for surgery and draped. A midline celiotomy incision was made starting from 2 cm cranial from the umbilicus to the pubic bone. Abdominal exploration confirmed a total vaginal prolapse with the uterine body and cervix disappearing in the remaining abdominal part of the vagina, and an additional herniation of the colon descendens and the bladder in the prolapsed vaginal tissue was seen. First, the urinary bladder and colon were placed back in their normal positions by gentle traction. Next, the prolapsed vaginal tissue was put back in its anatomical position by a combination of intra-abdominal pulling on the uterus and extra-abdominal

**Table 1. Complete blood count and biochemistry findings**

| Parameter | Value interval | Reference | Unit |
|---|---|---|---|
| WBC | 26.03* | 6.0-17.0 | m/mm$^3$ |
| Lym | 4.76 | 0.6-5.1 | m/mm$^3$ |
| Mon | 4.06 | 0.1-1.7 | m/mm$^3$ |
| Gra | 17.21 | 3.0-13.6 | m/mm$^3$ |
| RBC | 7.26 | 5.5-8.5 | m/mm$^3$ |
| PLT | 508 | 120-600 | m/mm$^3$ |
| **Electrolyte values** | | | |
| K | 3.2 | 3.7-5.6 | mEcq/L |
| Na | 149 | 141-153 | mEcq/L |
| Cl | 98 | 90-115 | mEcq/L |
| **Metabolite values** | | | |
| Glu | 82 | 55-102 | mg/dl |
| Lac | 0.7 | <2 | mmol/L |
| BUN | 37* | 5.600-11.80 | mg/dL |
| Creatinine | 3.6* | 0.500-1.500 | mg/dL |
| AST | 40 | 10.00-88.00 | U/L |
| Glucose | 108 | 60.00-110.0 | mg/dL |
| ALT | 29 | 10.00-88.00 | U/L |
| ALP | 52 | 22.00-150.0 | U/L |
| Magnesium | 1.5 | 1.200-2.000 | mg/dL |
| LDH | 81 | 50.0-495.0 | U/L |
| Total Bilirubin | 0.1* | 0.100-0.600 | mg/dL |
| Direct Bilirubin | 0.1 | 0.000-0.300 | mg/dL |
| Phosphorus | 4.9 | 2.200-5.500 | mg/dL |
| Cholesterol | 160 | 125.0-270.0 | mg/dL |
| Albumin | 2.8 | 2.300-3.800 | g/dL |
| Calcium | 9.4 | 8.600-11.20 | mg/dL |
| Triglycerides | 26 | 20.0-112.0 | mg/dL |
| Protein | 6.1 | 5.400-7.700 | g/dL |
| GGT | 1 | 1.000-10.00 | U/L |
| CPK | 207* | 20.00-200.0 | U/L |

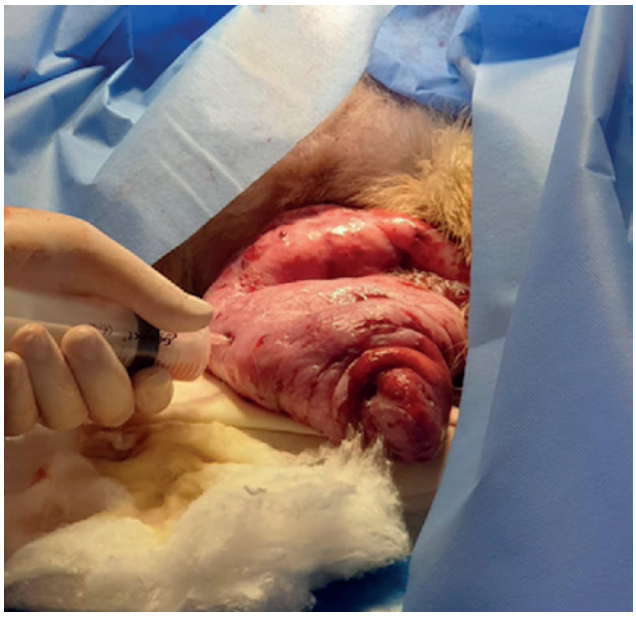


**Figure 3. Cystocentesis performed to empty the urinary bladder entrapped in the prolapsed tissues.**

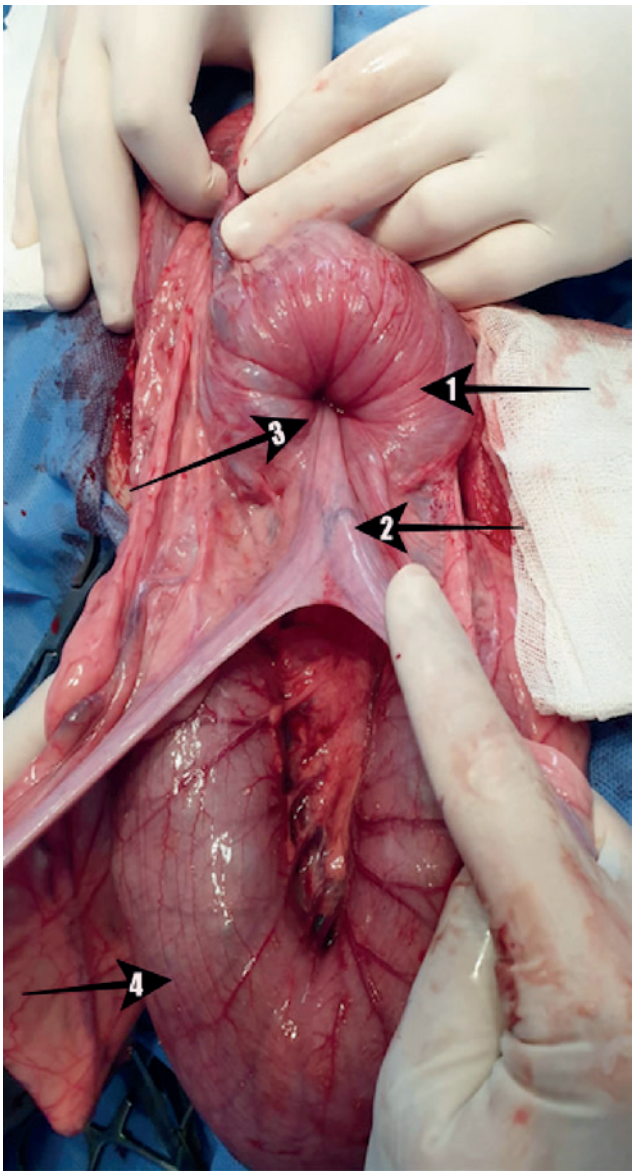



**Figure 4. After reduction of the vaginal prolapse, invagination of the uterine body and cervix remained. Note the overfilled colon due to obstruction from the previously herniated part. Arrow 1: cervix, arrow 2: uterus, arrow 3: invagination area, arrow 4: colon)**

pushing on the vaginal prolapse. Then, the invagination of the uterine body and cervix into the vagina was gradually corrected manually (Figure 4). Finally, standard ovariohysterectomy using USP 1 polyglycolic acid (Alcasorb, Katsan, Turkey) was performed. To prevent recurrence, cervicopexy was performed by anchoring the cervix to the ventral abdominal wall with USP 2/0 polyglycolic acid. The abdominal wall was closed with a simple continuous suture with USP 0 polyglycolic acid. Subcutaneous tissues were closed with a simple continuous suture with USP 0 polyglycolic acid. The skin was closed with interrupted horizontal mattress suture USP 0 silk (Alcasilk, Katsan, Turkey).

For postoperative analgesia, 0.2 mg/kg dose of meloxicam (Maxicam, Sanovel, Turkey) was administered on the day of surgery. In addition, 0.5 mg/kg ranitidine (Ulkuran Amp, Myfarma, Turkey) was given. A urinary catheter was placed and left for two days after the operation until the urinary output was normalized, vaginal swelling had lessened and the catheter was removed. Blood analysis performed on the second day after surgery showed blood urea nitrogen (BUN) and creatinine values were within the normal reference limits. After surgery, antibiotic treatment was continued with daily subcutaneous amoxicillin-clavulanic acid injections for seven days. The first three to four days after the surgery, there was vaginal discharge, which then decreased and ceased. After the sugery, the dog was hospitalized for seven days, after which the skin sutures were removed and the dog was discharged. Weekly control visits were performed. At the final follow-up one month after the operation, the dog had normal micturition without incontinence or straining. No other complications were observed (Figure 5).

## DISCUSSION

Urinary bladder retroflexion is a potentially life-threatening condition that occurs in both male and female dogs. In male dogs, bladder retroflexion is regularly seen in chronic perineal hernia (Sontas et al., 2008; Adeyanju et al., 2011). In female dogs, bladder retroflexion can occur as a rare complication of vaginal prolapse (Sontas et al., 2010; Acar et al., 2017). When bladder retroflexion leads to dysuria or anuria, the prognosis becomes grave to poor. Urethral obstruction can happen due to the external compression from swollen prolapsed vaginal tissue on the urethra, or when the vestibule prolapses together with the vagina leading to an external urethral orificium located on the ventral surface of the prolapsed tissue (Schaefers-Okkens, 2001; Sontas et al., 2010). Also herniation of the bladder into the vaginal prolapse might lead to urinary obstruction (Canatan et al., 2015). Urethral obstruction leads to acute kidney failure, recognized on blood analysis as postrenal azotemia (increased BUN and creatinine) and fast deterioration of the animal's general health condition (Niles and Williams, 1999). In the present case, it was thought that since the dog was not able to urinate because of urethral obstruction, the serum BUN and creatinine concentrations increased.

Emergency treatment consists of fluid treatment and establishing a patent urinary tract (Sontas et al., 2008). Since this is often not possible, early operative treatment of vaginal prolapse is recommended (Canatan et al., 2015). To improve the metabolic status of the dog in the present case, fluid therapy was installed and the bladder was emptied by cystocentesis before she was brought under general anesthesia.

Chronic straining and increased abdominal pressu-

re due to irritation of prolapsed vaginal tissue lead to worsening of the vaginal prolapse (Alan et al., 2007), which may become further complicated by the herniation of neighboring structures. Because of their close association and ligamentous connections, the colon descendens and the urinary bladder may become involved in the vaginal prolapse (Ober et al., 2016). In the present case, the urinary bladder was detected in the prolapsed tissue before surgery. However, the additional cervical invagination in the vagina and herniation of the colon descendens were only recognized during abdominal exploration.

Repositioning of incarcerated organs may lead to the release of toxins from these vascularly compromised tissues (Sontas et al., 2010). Therefore, the vaginal mucosa was carefully inspected, disinfected, and surgically debrided before surgical repositioning was attempted. During the abdominal exploration, the regional vasculature to the vagina was inspected. If the vaginal arterial supply would have been torn or the venous drainage thrombozed, vaginectomy would have been necessary (Prassinos et al., 2010). During abdominal retraction and repositioning of the retroflexed urinary bladder and the herniated descending colon, no major tissue trauma was identified.

Cystopexy can be performed in dogs to prevent the recurrence of bladder retroflexion (Rawlings et al., 2002). In this case, it was assumed that direct traction of the bladder into the prolapsed vagina was the cause of its retroflexion. Since the vaginal prolapse was reduced and recurrence was prevented by cervicopexy, cystopexy was not deemed necessary.

In this presented case, ovariohysterectomy was performed to eliminate future hormonal effects causing potential recurrence of the vaginal prolapse. But at the same time, it also prevented a potential recurrence of the cervical invagination into the vagina.

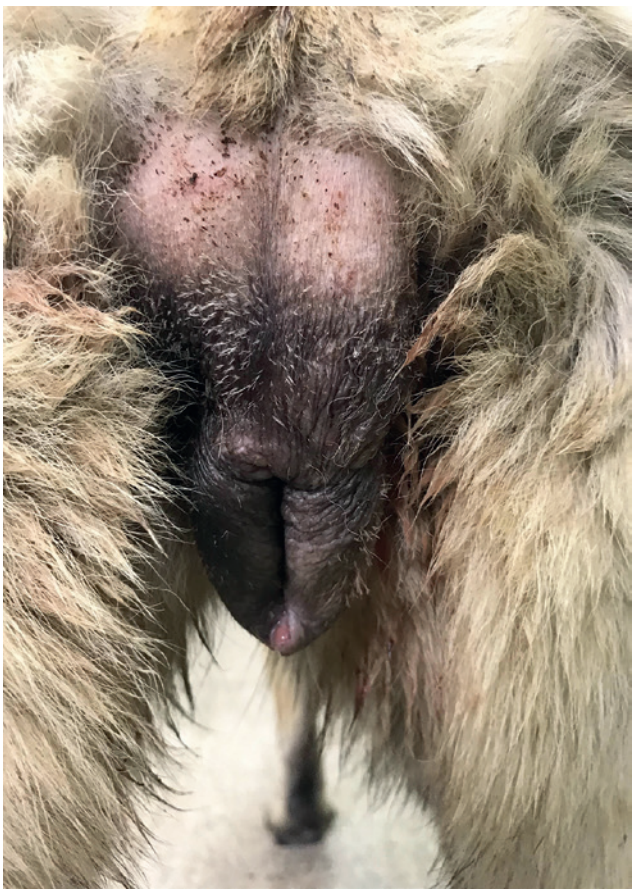


**Figure 5. Normal aspect of the vulva at seven days after surgery.**

**Table 2. Blood gas findings.**

| Parameter | Value interval | Reference | Unit |
|---|---|---|---|
| **Blood Gas** | | | |
| pH | 7.365 | 7.33-7.44 | mmHg |
| $pCO_2$ | 32.5 | 35-42 | mmHg |
| $pO_2$ | 41.2 | 73-92 | mmHg |
| **Acid-Base Balance** | | | |
| $cBase\ (Ecf)_c$ | -6.8 | (-8.65)–(-5.30) | mmol/L |
| $cBase\ (B)_c$ | -5.9 | (-6.25)–(-3.15) | mmol/L |
| $cHCO_3^-(P,st)_c$ | 19.1 | 23-27 | mmol/L |
| $cHCO_3^-(P)_c$ | 18.6 | 16.40-19.45 | mmol/L |

## CONCLUSION

In conclusion, it should be taken into consideration that vaginal prolapse complicated with urinary bladder retroflexion is most frequently seen in large breed dogs. Such cases progress with the development of dysuria and uremia, which worsen the prognosis in time; an urgent surgical treatment option should always be considered. Furthermore, it was concluded that ultrasonographic examination contribute to the early diagnosis, differential diagnosis and prognosis of animals that have complications along with the vaginal prolapse.

## REFERENCES

Acar D. B., Birdane M. K. (2017). Urinary bladder retroflexion and dystocia in a bitch with the vaginal supportive connective tissue failure. *Ankara Üniversitesi Veteriner Fakültesi Dergisi 64*, 61-64.

Adeyanju J. B., Yakubu A. S., Jibril A., Buhari S., Alayande M. O. (2011). Bilateral perineal hernia with bladder retroflexion in a 13 year old intact Jack-Russel Dog: Case report. *Sokoto Journal of Veterinary Sciences 9*, 50-54.

Alan M., Cetin Y., Sendag S., Eski F. (2007). True vaginal prolapse in a bitch. *Animal Reproduction Science 100*, 411-414.

Canatan H. E., Ergin I., Polat I. M., Yazlik M. O. (2015). Unusual cases of vaginal prolapse concurrent with cystocele in two dogs. *Revue de Médecine Vétérinaire 166*, 43-46.

Feldman E. C., Nelson R. W. (2004). Vaginal hyperplasia / Vaginal prolapse. In: Feldman E. C., Nelson R. W. (editors). *Canine and Feline Endocrinology and reproduction*. Third edition, Missouri, Saunders, p. 906-909.

Gouletsou P. G., Galatos A. D., Apostolidis K., Sideri A. I. (2009). Vaginal fold prolapse during the last third of pregnancy, followed by normal parturition, in a bitch. *Animal Reproduction Science 112*, 371-376.

Johnston S. D., Kustritz M. V., Olson P. S. (2001). *Canine and Feline Theriogenology*. First edition, London, Saunders, p. 225-273.

Nak D., Kaşıkçı G. (2013). İnfertilite, prolapsus vajina. In: Kaymaz M., Fındık M., Rişvanlı A., Köker A. (editors). *Köpek ve Kedilerde Doğum ve Jinekoloji*. First edition, Malatya, Medipres, p. 254-259.

Niles J. D., Williams J. M. (1999). Perineal hernia with bladder retroflexion in a female cocker spaniel. *Journal of Small Animal Practice 40*, 92-94.

Ober C. A., Petean C. P., Bel L. V., Taulescu M., Cătoi C., Bogdan S., Milgram J., Schwarz G., Oana L. L. (2016). Vaginal prolapse with urinary bladder incarceration and consecutive irreducible rectal prolapse in a dog. *Acta Veterinaria Scandinavica 58*, 54.

Özenç E., Birdane M. K., Yaprakçı M. V., Demirkan İ. (2017). Bladder retroflexion in a pointer bitch during pregnancy. *Kafkas Universitesi Veteriner Fakültesi Dergisi 23*, 513-514.

Prassinos N., Adamama-Moraitou K., Ververidis H., Anagnostou T., Kladakis S. (2010). Vaginal rupture and evisceration in a dog. *Acta Veterinaria Hungarica 58*, 309-315.

Rawlings C. A., Howerth E. W., Mahaffey M. B., Foutz T. L., Bement S., Canalis C. (2002). Laparoscopic-assisted cystopexy in dogs. *American Journal of Veterinary Research 63*, 1226-1231.

Sarrafzadeh-Rezaei F., Saifzadeh S., Mazaheri R., Behfar M. (2008). First report of vaginal prolapse in a bitch treated with oestrogen. *Animal Reproduction Science 106*, 194-199.

Schaefers-Okkens A. C. (2001). Vaginal edema and vaginal fold prolapse in the bitch, including surgical management. In: Concannon PW, England G, Verstegen J, (editors). *Recent Advances in Small Animal Reproduction. International Veterinary Information Service*, Ithaca, New York, http://www.ivis.org/advances/concannon/schaefers/chapter%20frm.asp?FLA=1 Date of Access: 12.12.2019

Sontas H. B., Apaydin S. Ö., Toydemir T. S. F., Kasikci G., Ekici H. (2008). Perineal hernia because of retroflexion of the urinary bladder in a rottweiler bitch during pregnancy. *Journal Small Animal Practice 49*, 421-425.

Sontas H. B., Ekici H., Romagnoli S. (2010). Canine vaginal fold prolapse: a comprehensive literature review. *European Journal of Companion Animal Practice 20*, 127.

White R. A. S., Herrtage M.E. (1986). Bladder retroflexion in the dog. *Journal Small Animal Practice 27*, 735-746.

# Ventral rhinotomy to remove a nasopharyngeal polyp that invaded the frontal sinus in a cat

*Ventrale rinotomie ter verwijdering van een nasofaryngeale poliep met extensie in de frontale sinus bij een kat*

**[1]R. Jacobs, [1]B. Van Goethem, [2]E. Stock, [3]H. De Cock, [1]T. Bosmans, [1]H. de Rooster**

[1]Small Animal Department, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium
[2]Department of Medical Imaging of Domestic Animals, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium
[3]AML/Veterinary Pathology Services, Emiel Vloorsstraat 9, 2020 Antwerpen, Belgium

bart.vangoethem@ugent.be

## ABSTRACT

**A six-year-old, male, castrated European shorthair cat was presented due to a lifelong history of sneezing, nasal discharge, open-mouth breathing, stertor and vestibular problems. Clinical examination showed absence of airflow through the nostrils. Computed tomography of the head revealed an infiltrative soft tissue attenuating mass in the left nasal cavity, nasopharynx and the left frontal sinus. All of these locations could be successfully accessed through ventral rhinotomy and the mass was completely removed. Histological examination identified it as an inflammatory polyp. All respiratory and neurological symptoms disappeared, and two-and-a-half years after surgery, the cat was still without complaints.**

## SAMENVATTING

Een zes jaar oude, mannelijke, gecastreerde Europese korthaar werd aangeboden wegens een levenslange voorgeschiedenis van niezen, neusvloei, open-mondademen, stertor en vestibulaire problemen. Klinisch onderzoek toonde de afwezigheid van luchtstroom aan doorheen de neusgaten. Computertomografie van het hoofd onthulde een infiltratieve wekedelenmassa in de linkerneusholte, nasofarynx en de linker frontale sinus. Deze drie locaties konden succesvol worden bereikt door middel van ventrale rinotomie en de massa werd volledig verwijderd. Via histologisch onderzoek werd de massa geïdentificeerd als een inflammatoire poliep. Alle respiratoire en neurologische symptomen verdwenen en tweeënhalf jaar na de operatie was de kat nog steeds vrij van klachten.

## INTRODUCTION

In cats younger than two years of age, a mass in the nasopharynx is most commonly an inflammatory polyp (Allen et al., 1999). These non-neoplastic growths arise from the lining of the auditory tube or the middle ear (Reed and Gunn-Moore, 2012). They either grow into the tympanic cavity, potentially extending into the ear canal (middle ear polyps) or they grow towards the nasopharynx (nasopharyngeal polyps) (Muilenburg and Fry, 2002; Reed and Gunn-Moore, 2012). Polyps most commonly occur unilaterally, but bilateral nasopharyngeal polyps as well as the combination of a nasopharyngeal and an aural polyp have been reported (Muilenburg and Fry, 2002; MacPhail et al., 2007).

Treatment of a nasopharyngeal polyp includes traction avulsion of the polyp from an oral approach, followed by anti-inflammatory medication to decrease mucosal swelling and resolve auditory tube obstruction (Anderson et al., 2000). In rare cases, it is necessary to incise the soft palate for additional surgical exposure (Kudnig, 2002).

In this case report, a ventral rhinotomy approach is described for a large nasopharyngeal polyp that also filled the left nasal conchae and even extended into the left frontal sinus.

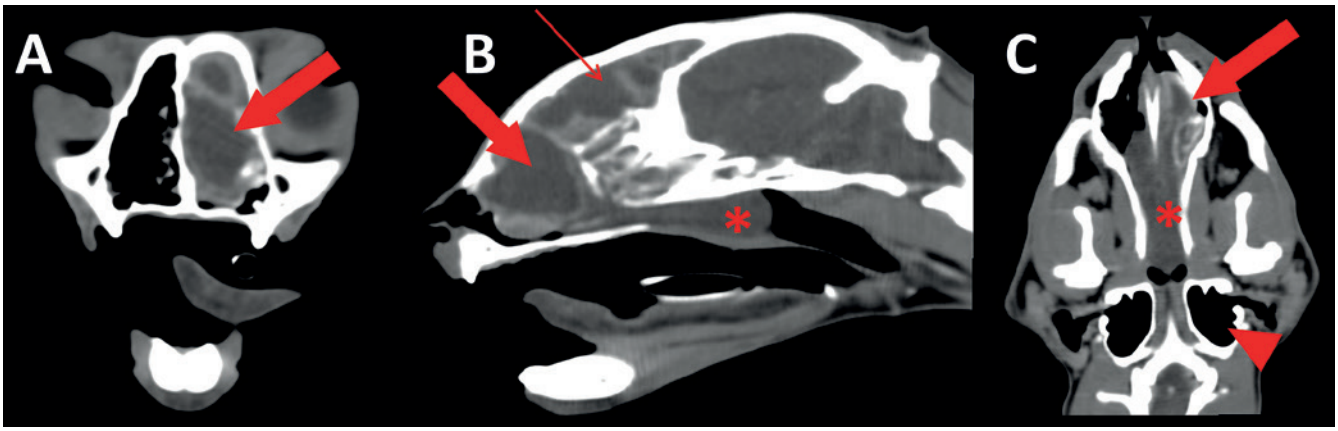



**Figure 1. CT images of the head. A. Transverse view showing the polyp in the left nasal cavity (large arrow). B. Sagittal view showing the polyp in the left nasal cavity (large arrow), the frontal sinus (small arrow) and the oropharynx (asterisk). C. Dorsal view showing the polyp in the left nasal cavity (large arrow) and the oropharynx (asterisk). Also note the normal air-containing bulla (arrowhead).**

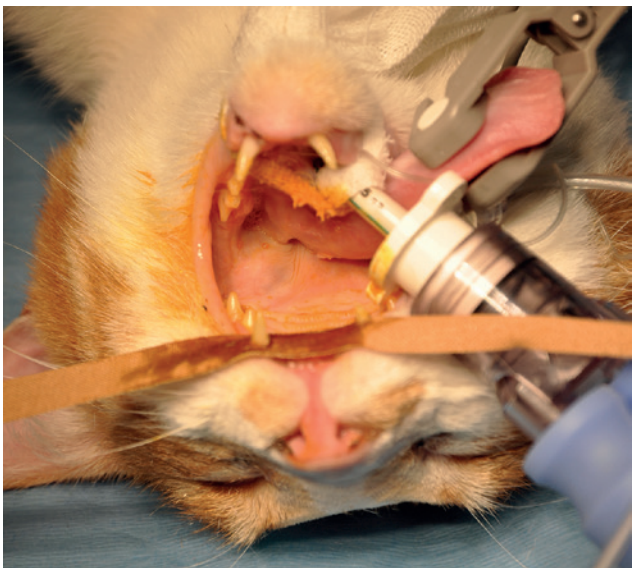


**Figure 2. Patient in dorsal recumbency with the tongue and endotracheal tube lateralized to the right. Submaximal opening of the mouth allowed sufficient access to perform a ventral rhinotomy.**

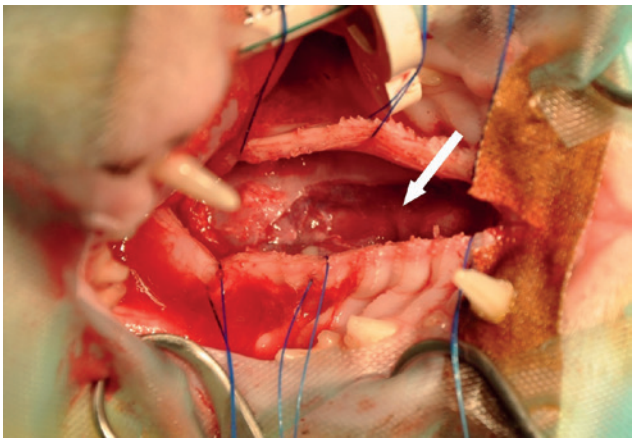


**Figure 3. After incising the hard palate mucosa in the midline and retracting it with stay sutures, the left palatal bone was partially removed to allow access to the left nasal cavity. This easily revealed the intranasal part of the polyp (arrow).**

## CASE HISTORY

A six-year-old, male, castrated European shorthair cat was presented because of a lifelong history of sneezing, chronic intermittent purulent nasal discharge, open-mouth breathing, stertor and partial anorexia. In the past, antibiotics and corticosteroids provided temporary improvement, but recently the anorexia had become problematic.

At general examination, open-mouth breathing and the absence of airflow through the nostrils were obvious. All other clinical parameters were normal. There were no neurological signs and CBC and biochemistry were unremarkable. FIV/FeLV testing was negative. Likewise, radiographs of the thoracic cavity and abdomen, abdominal ultrasonography and urine analysis, all performed to identify the cause of the partial anorexia, failed to disclose any pathology. A CT scan of the head was performed and revealed a soft tissue attenuating mass in the nasopharynx. The mass was ill-defined rostrally and extended into the left nasal passage, with destruction of the turbinates, and also grew into the left frontal sinus (Figure 1). There was moderate peripheral ring-like contrast enhancement. A minimal amount of non-contrast enhancing soft tissue attenuating material was present in the left tympanic bulla, the bulla wall was within normal limits. There was no invasion of the cribriform bone and no regional lymphadenopathy.

Rhinoscopic biopsies consisted of a core of loosely fibrovascular tissue bordered by ciliated and partially stratified epithelium, propria edema and neovascularization associated with a diffuse inflammatory cell infiltrate, all indicative of an inflammatory polyp.

After institution of general anesthesia and adequate multimodal analgesia, the cat was positioned in dorsal recumbency with the tongue and endotracheal tube lateralized to the right side and the mouth opened with a non-spring-loaded mouth gag (Figure 2). A midline incision was made in the mucosa overlying the hard palate continuing halfway the soft

palate. With a periosteal elevator, the mucosa was released from the underlying bone and several stay sutures were placed at the mucosal borders. Hemostasis was achieved with counter pressure and judicious use of bipolar electrocoagulation forceps. Using a 5 mm neurosurgical drill, a 15x40 mm hole was made in the hard palate overlying the left nasal cavity (Figure 3).

After removal of copious amounts of mucus from the left nasal cavity, the visible part of the polyp was gently lifted. Further traction also freed the part in the frontal sinus (Figure 4). The polyp appeared to be only attached in the region of the auditory tube; yet, after extraction, no visible stalk could be recognized. The mucosa surrounding the auditory tube exit was therefore curetted. The frontal sinus was abundantly flushed by passing a tube through the enlarged sinonasal foramen. Any damaged nasal turbinates were removed and patency of the nasal cavity was confirmed by passing a feeding tube through the left nostril. No attempt was made to close the bony defect in the palate. The oral mucosa was closed with simple interrupted 5/0 polyglecaprone (Monocryl, Ethicon, Diegem) sutures (Figure 5).

Histological examination of the mass was in accordance with the previous biopsy results and confirmed the presence of an inflammatory polyp.

The cat was hospitalized for two days during which he made an uncomplicated recovery. The cat was reluctant to eat the first day but was eagerly eating on the second day. Postoperative analgesia consisted of opioids for two days and NSAIDs for seven days. Broad-spectrum antibiotics were also given for seven days.

At the time of the follow-up visit fourteen days later, both nasal passages were patent. Oral inspection confirmed uncomplicated healing of the palatal mucosa. The owner reported that the cat's appetite had completely normalized and that he was enthusiastically smelling again his surroundings. The cat was examined again ten months postoperatively and the owner was contacted by telephone two and a half years after the procedure. No recurrence of any upper respiratory symptoms was ever observed.

## DISCUSSION

A nasopharyngeal polyp that extended in the nasal cavity and frontal sinus could be successfully removed using a ventral rhinotomy approach. Ventral rhinotomy in cats has only been sparsely reported (Holmberg et al., 1989; Holmberg, 1996). Nevertheless, it is a very useful approach to the nasal cavity since it allows direct access to the ventral nasal cavity and the nasopharynx. Postoperatively, there is no visible scar and no risk for development of subcutaneous emphysema, which is common after dorsal rhinotomy (Weeden and Degner, 2016). An uncommon complication after ventral rhinotomy might be the development of an oronasal fistula, leading to chronic nasal infection and subsequent nasal discharge (Holmberg, 1996).

The standard treatment of an oropharyngeal polyp is traction avulsion from an oral approach with forces directed towards the oral cavity (Anderson et al., 2000). This technique is successful in removing not only the polyp but also often its originating stalk (Anderson et al., 2000; Lanz and Wood, 2004). Based on the CT images, it was deemed highly unlikely that this technique would be successful in the present case. The dimensions of the parts of the polyp present in the nasal cavity and frontal sinus prevented its passage through the nasopharynx.

Generally, a ventral rhinotomy does not allow access to the frontal sinuses (Weeden and Degner, 2016). However, based on the amount of ethmoturbinate destruction and bony lysis at the sinonasal junction seen on the preoperative CT images, it was assumed that a ventral rhinotomy would actually grant access to both

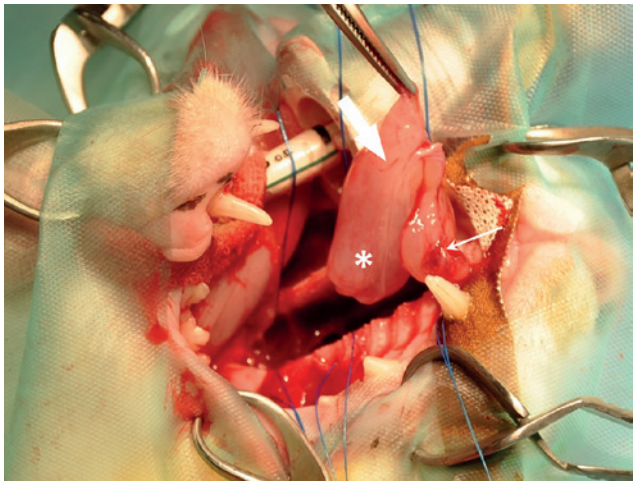


**Figure 4. Gentle traction avulsion resulted in removal of all parts of the polyp: the ventral part present in the nasal cavity (large arrow) and the oropharynx (asterisk) and the dorsal part present in the frontal sinus (small arrow).**

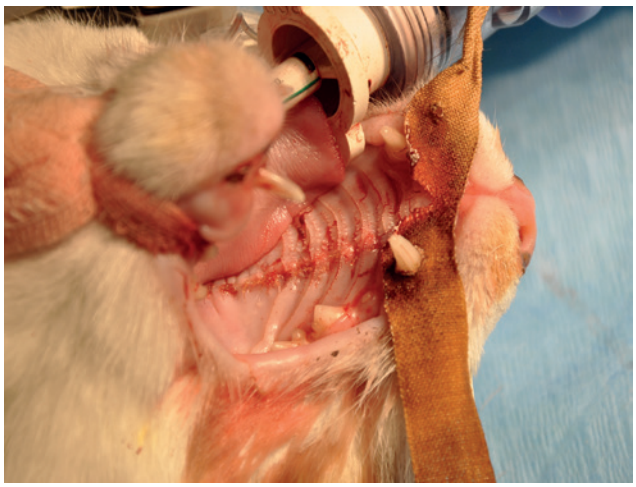


**Figure 5. The oral mucosa was closed with simple interrupted sutures polyglecaprone 5/0. No attempt was made to first close the underlying bony defect.**

the nasal and frontal sinus in this particular case. Intraoperatively, the frontal sinus could be adequately visualized to confirm complete removal of the polyp, while, at the same time, the pharyngeal orifice of the auditory tube could be reached.

The ventral rhinotomy approach necessitates adequate opening of the mouth for visualization and access for a prolonged period. This can have grave consequences since the maxillary artery in cats is the sole artery responsible for cerebral perfusion and could become compressed between the tympanic bulla and the angular process of the mandibula (Barton-Lamb et al., 2013). The resulting cerebral hypoxia leads to neurologic signs, such as cortical blindness, motoric impairment or even respiratory and cardiac arrest (Hartman, 2017). It is therefore essential not to open the mouth maximally, nor to use spring-loaded mouth gags. The latter can unwillingly achieve maximal mouth opening during the procedure (Martin-Flores et al., 2014).

For various reasons including advanced age, extension into the frontal sinus and destruction of turbinates, a nasopharyngeal polyp was not the most likely tentative diagnosis in the cat of the present case (Reed and Gunn-Moore, 2012). The most common cause of nasopharyngeal disease in older cats is neoplasia, with lymphoma (29-70%) and adenocarcinoma (13-15%) being the most common tumors. Benign nasopharyngeal polyps are considered uncommon (Henderson, 2004; Worley, 2016). Yet, age is not a reliable parameter to differentiate since nasal tumors have been reported in cats ranging between three and seventeen years and, for nasopharyngeal polyps, three months to fifteen years has been recorded (Reed and Gunn-Moore, 2012).

Inflammatory polyps of the nasal turbinates, currently referred to as nasal chondromesenchymal hamartoma, were an important differential in this case. They arise from the nasal turbinates themselves and therefore have a more rostral location than nasopharyngeal polyps (Grecci et al., 2011). Radiographic studies typically demonstrate soft tissue opacification of the nasal cavity, turbinate lysis and radiolucent areas, corresponding to cystic spaces within the lesion (Grecci and Montellaro, 2016). In addition, their histological appearance is very distinct, characterized by fibrovascular tissue lined by a stratified squamous or ciliated columnar epithelium and bony cartilage structures without signs of atypia and erythrocyte-filled spaces (Grecci et al., 2011). Because of their location in the rostral nasal cavity surgical removal necessitates rhinotomy (Weeden and Degner, 2016).

It can be concluded that ventral rhinotomy in this cat resulted in adequate access to successfully remove a sizeable nasopharyngeal polyp that caused turbinate destruction and extended into the frontal sinus.

## REFERENCES


Allen H.S., Broussard J., Noone, K. (1999). Nasopharyngeal diseases in cats: a retrospective study of 53 cases. *Journal of the American Veterinary Medical Association 35*, 457-461.

Anderson D.M., Robinson R.K., White R.A.S. (2000). Management of inflammatory polyps in 37 cats. *Veterinary Record 147*, 684-687.

Barton-Lamb A.L., Martin-Flores M., Scrivani P.V., Bezuidenhout A.J., Loew E., Erb H.N., Ludders J.W. (2013). Evaluation of maxillary arterial blood flow in anesthetized cats with the mouth closed and open. *Veterinary Journal 196*, 325-331.

Greci V., Mortellaro C.M., Olivero D., Cocci A., Hawkins E.C. (2011). Inflammatory polyps of the nasal turbinates of cats: an argument for designation as feline mesenchymal nasal hamartoma. *Journal of Feline Medicine and Surgery 13*, 213-219.

Greci V., Mortellaro C.M. (2016). Management of otic and nasopharyngeal, and nasal polyps in cats and dogs. *Veterinary Clinics of North America: Small Animal Practice 46*, 643-661.

Hartman E.A., McCarthy R.J., Labato M.A. (2017). Global cerebral ischemia with subsequent respiratory arrest in a cat after repeated use of a spring-loaded mouth gag. *Journal of Feline Medicine and Surgery Open Reports 27*, 2055116917739126.

Henderson S.M., Bradley K., Day M.J., Tasker S., Caney S.M., Hotston Moore A., Gruffydd-Jones T.J. (2004). Investigation of nasal disease in the cat-a retrospective study of 77 cases. *Journal of Feline Medicine and Surgery 6*, 245-257.

Holmberg D.L. (1996). Sequelae of ventral rhinotomy in dogs and cats with inflammatory and neoplastic nasal pathology: A retrospective study. *The Canadian Veterinary Journal 37*, 483-485.

Holmberg D.L., Fries C., Cockshutt J., Van Pelt D. (1989). Ventral rhinotomy in the dog and cat. *Veterinary Surgery 18*, 446-449.

Kudnig S.T. (2002). Nasopharyngeal polyps in cats. *Clinical Techniques in Small Animal Practice 17*, 174-177.

Lanz O.I., Wood B.C. (2004). Surgery of the ear and pinna. *Veterinary Clinics: Small Animal Practice 34*, 567-599.

MacPhail C.M., Innocenti C.M., Kudnig S.T., Veir J.K., Lappin M.R. (2007). Atypical manifestations of feline inflammatory polyps in three cats. *Journal of Feline Medicine and Surgery 9*, 219-225.

Martin-Flores M., Scrivani P.V., Loew E., Gleed C.A., Ludders J.W. (2014). Maximal and submaximal mouth opening with mouth gags in cats: implications for maxillary artery blood flow. *Veterinary Journal 200*, 60-64.

Muilenburg R.K., Fry T.R. (2002). Feline nasopharyngeal polyps. *Veterinary Clinics of North America: Small Animal Practice 32*, 839-849.

Reed N., Gunn-Moore D. (2012). Nasopharyngeal disease in cats: 1. Diagnostic investigation. *Journal of Feline Medicine and Surgery 14*, 306-315.

Reed N., Gunn-Moore D. (2012). Nasopharyngeal disease in cats: 2. Specific conditions and their management. *Journal of Feline Medicine and Surgery 14*, 317-326.

Weeden A.M., Degner D.A. (2016). Surgical approaches to the nasal cavity and sinuses. *Veterinary Clinics of North America: Small Animal Practice 46(4)*, 719-733.

Worley D.R. (2016). Nose and nasal planum neoplasia, reconstruction. *Veterinary Clinics of North America: Small Animal Practice 46*, 735-750.

# Duodenal peripapillary adenocarcinoma causing extrahepatic biliary obstruction and icterus in a cat

*Duodenaal peripapillair adenocarcinoom leidend tot extrahepatische galobstructie en icterus bij een kat*

**[1]T. Rick, [1]E. Stock, [2]I. Van de Maele, [3]E. Kammergruber, [1]J. Saunders**

[1]Department of Medical Imaging of Domestic Animals and Orthopedics of Small Animals
[2]Department of Small Animals
Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium
[3]Department of Pathobiology, Faculty of Veterinary Medicine, Utrecht University,
Yalelaan 1, CL-3584 Utrecht, the Netherlands

thorsten.rick@ugent.be

## ABSTRACT

**A six-year-old, female, neutered domestic shorthair cat was presented with chronic weight loss and a two-day history of partial anorexia and lethargy. Abdominal ultrasonography revealed a regional thickening of the duodenal wall with loss of normal layering, a normally walled segmentally dilated distal aspect of the common bile duct containing slightly hyperechoic bile, and a mild to moderately enlarged major duodenal papilla. Based on the ultrasound examination, the primary differential diagnosis was a peripapillary duodenal neoplastic or less likely, an inflammatory or infectious process with secondary extrahepatic biliary obstruction. Postmortem examination revealed a duodenal, peripapillary adenocarcinoma with metastasis into the liver and lymph nodes, and external compressive obstruction of cystic- and common bile duct.**

## SAMENVATTING

Een zes jaar oude, vrouwelijke, gesteriliseerde Europese korthaar werd aangeboden voor chronisch vermageren, en partiële anorexie en lethargie die reeds twee dagen aanwezig waren. Op abdominale echografie was er een focale verdikking van de wand van het duodenum met verlies van het normale patroon van de lagen. De gemeenschappelijke galafvoergang was focaal gedilateerd en bevatte echogene gal. De papilla duodenalis was mild tot matig gedilateerd. Op basis van het echografisch onderzoek was de meest waarschijnlijke diagnose een peripapillaire duodenale neoplasie, of minder waarschijnlijk, een inflammatoir of infectieus proces, met secundaire extrahepatische galgangobstructie. Tijdens autopsie werden een duodenaal peripapillair adenocarcinoom met metastasen in de lever en regionale lymfeknopen, en een obstructie van de extrahepatische galgangen gediagnosticeerd.

## INTRODUCTION

Extrahepatic biliary obstruction is a well-known complication in feline veterinary medicine and can be caused by mural thickening, luminal obstruction or extraluminal compression. There are some reports in cats, including inflammatory and parasitic infectious diseases (common bile duct, liver, pancreas or duodenum), neoplastic processes (originating from the common bile duct, the liver, the pancreas or the major duodenal papilla) and reports describing extrahepatic biliary obstruction secondary to internal occlusion such as plugs, cholelithiasis or foreign bodies. Extrahepatic biliary obstruction has also been described secondary to congenital malformations, diaphragmatic hernia, liver fluke infestation and cysts. Non-obstructive dilation of the common bile duct has been reported as well and can be caused by segmental dilation or choledochal cysts (D´Anjou and Penninck, 2015; Gaillot et al., 2007; Head and Daniel, 2005; Lawrence et

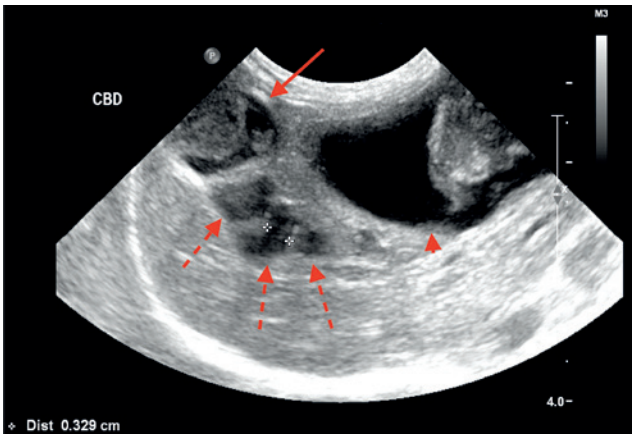



**Figure 1. Long axis ultrasound image of the gallbladder (long arrow), the non-dilated, tortuous common bile duct (dashed arrow) and the segmental dilatation of the common bile duct (short arrow). Note the severe segmental dilation of the distal common bile duct compared to the middle part.**

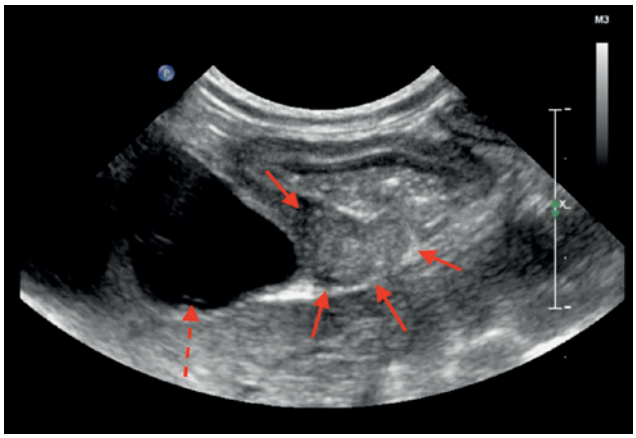


**Figure 2. Long axis image of the duodenum in the region of the major duodenal papilla. The papilla is moderately enlarged (solid arrows) and there is segmental dilation of the distal common bile duct adjacent to it (dashed arrow).**

al., 1992; Mayhew et al., 2002; Neer, 1992; Gaschen, 2011; Kelly et al., 1975; Mayhew and Weisse, 2008; Barsanti et al., 1976; Haines et al., 1996; Brioschi et al., 2014; Naus and Jones 1978; Della Santa et al., 2007; Spain et al.,2017; Grand et al., 2018).

Extrahepatic biliary obstruction can be challenging to diagnose since the history and clinical signs are often unspecific, including icterus, nausea, decreased appetite and vomiting. Blood examination often reveals hyperbilirubinemia and a marked increase in serum alkaline phosphatase activity but does not lead to final conclusions (Head and Daniel, 2005). Ultrasonography is considered to be the modality of choice for the diagnosis of extrahepatic biliary obstruction in veterinary medicine. However, the detection of specific lesion arrangement, such as focal mass or the distinction between obstructive or non-obstructive dilation of the common bile duct can be challenging (Gaillot et al., 2007). In a study by Gaillot et al. (2007), describing thirty cats with extrahepatic biliary obstruction, the pancreatic origin of the triggering mass was identified in 55 % of the cats; in just 18 % of these masses the dignity was accurately recognized by ultrasound (Fahle et al.,1995; Leveille et al., 1996; Gaillot et al., 2007; Brioschi et al., 2014; Spain et al., 2017).

In human medicine, the most common causes of extrahepatic biliary obstruction are choledocholithiasis and pancreatic carcinoma (Materne et al., 2000). Assessment of human partial or complete obstruction is based on clinical, laboratory, ultrasonographic and endoscopic parameters (Bakaeen et al., 2000; Kim et al., 2001). To the authors' best knowledge, this is the first report describing the imaging findings in an extrahepatic biliary obstruction caused by a duodenal peripapillary adenocarcinoma in a cat.

## CASE DESCRIPTION

A six-year-old, female, neutered domestic shorthair cat was presented with weight loss for already a few months and a two-day history of partial anorexia and lethargy. The clinical examination was characterized by a reduced body condition score of 2/9, mild discomfort at abdominal palpation and jaundice. Hematogeneous examination revealed the following abnormal blood values: hyperbilirubinemia 12.8 mg/dL (reference range < 0.40 mg/dL), elevated aspartate transaminase 116 U/L (reference range <83 U/L), alanine transaminase 328 U/L (reference range <91 U/L), gamma glutamyltransferase 42 U/L (reference range <10 U/L) and alkaline phosphatase 217 U/L (reference range 17-63 U/L). At this point, neoplasm with hepatobiliary origin, inflammation and/or infection affecting the bile duct system were hypothesized. Abdominal ultrasonography was performed using an 8-5 MHz broadband curved array transducer and a 12-5 MHz linear array transducer (iU22 Philips, Bothell, WA). Ultrasonographic examination revealed irregular, thickened and diffusely hyperechoic mesenteric fat in the cranial abdomen. The liver was subjectively enlarged and demonstrated a heterogeneous, slightly hyperechoic parenchyma. Multiple, small, anechoic tubes with hyperechoic wall and absent Doppler signal corresponding to dilated intrahepatic bile ducts were detected. The gallbladder was moderately filled with bile and a moderate to large amount of organized, hyperechoic material attached to the dependent wall. The common bile duct demonstrated a tortuous course with mild amount of intraluminal sludge but still had a normal luminal diameter (3.4 mm). Proximal to the major duodenal papilla, there was a focal, fluid filled, segmental dilation of the distal aspect of the common bile duct present (2.2 x 3.0 cm; height x length) (Fig-

ure1). The wall of the common bile duct was folded and partially protruded into the luminal widening. The major duodenal papilla was mildly to moderately enlarged (11.0 mm in diameter) (Figure 2). The adjacent portion of the duodenum presented a focal, moderate transmural thickening of the wall (9.4 mm), with diffuse pseudolayering and a concurrent hypoechoic, ill-defined focal asymmetric thickening of the muscularis layer proximal to the duodenal papilla (16 mm) (Figure 3). There was no peristalsis detected in this portion of the duodenum. The stomach was moderately filled with food, although the cat had not been fed during the last eight hours. The pancreas was subjectively normal in size with well-defined demarcation towards the duodenum and showed a hypoechoic parenchyma with a moderate dilation of the pancreatic duct (diameter of 2.5 mm). The gastric and pancreaticoduodenal lymph nodes were mildly enlarged (6 mm), preserving normal echogenicity and shape.

Based on the ultrasound findings, the clinical presentation and laboratory test results, the remaining differential diagnosis included extrahepatic biliary tract obstruction secondary to a duodenal and papillary infiltrate of inflammatory or neoplastic origin with lymphatic metastasis or reactive adenitis, regional steatitis and gastroduodenal subobstruction. Ultrasound guided fine-needle aspiration of the pancreaticoduodenal lymph nodes showed low cellularity; the nodes were not diagnostic. Surgical exploration and sampling of the lesion were advised with potential medical or surgical treatment in form of a choledochoduodenostomy and stent placement. Based on financial concerns and the suspicion of a neoplastic process, the owner declined any further examinations and the cat was euthanized and submitted for necropsy.

Postmortem examination grossly revealed a diffuse segmental dilation of the bile duct filled with increased viscous bile. Surrounding the major papilla, the duodenal wall was irregularly thickened over a length of 4 cm and was subjectively firm on palpation (Figure 4). The duodenal mucosa showed few ulcerations. The regional lymph nodes were moderately enlarged. Furthermore, within the hepatic parenchyma, there were two well-circumscribed whitish nodular areas within the left caudal (1.0 cm in diameter) and right cranial lobe (0.5 cm in diameter) detectable.

Histologic evaluation of the duodenum showed a multi-lobulated neoplasm transmurally affecting the duodenal wall with focal infiltration of the major duodenal papilla. The neoplastic cells formed multifocal acini and tubuli lined by cuboidal cells with large, round, centrally located nuclei and moderate amount of eosinophilic cytoplasm. Some acini contained mucous material. A large amount of fibrovascular stroma was supporting the neoplastic cells. Multifocal areas of necrosis and ulceration accompanied those areas, and neoplasm was seen at the mucosal epithelium. The mitotic figure was one per high power field of 40x. Histologic evaluation of the hepatic nodules and enlarged lymph nodes revealed infiltrative secondary neoplastic tissue representing the same characteristics as the aforementioned duodenal neoplasm (consistent with metastasis). Within portal areas, bile duct proliferation, fibrosis and inflammation (lymphoplasmacytic) were present. In some areas, the fibrosis extended in between the hepatocytes (dissecting fibrosis).

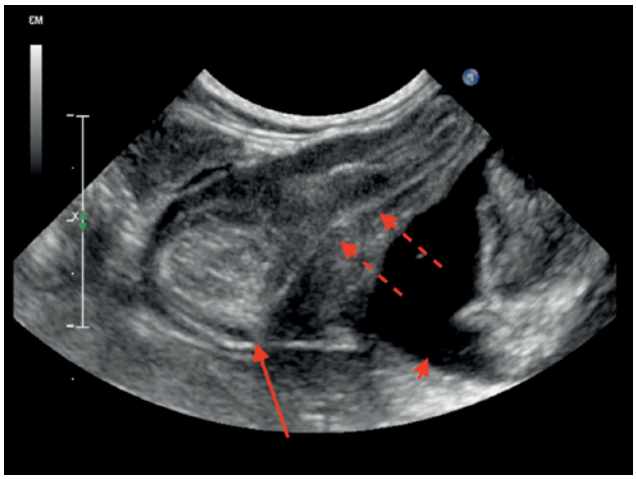



**Figure 3. Ultrasound image in oblique axis of the transition zone between abnormal and normal duodenal tissue. Proximal is to the left. There is mild luminal dilation present in the proximal aspect (solid arrow) and loss of normal wall layering distal to it (dashed arrows). There is normalization of the duodenal wall layering in the distal aspect on the left of the image. The segmental dilation of the common bile duct is adjacent to it (arrowhead).**

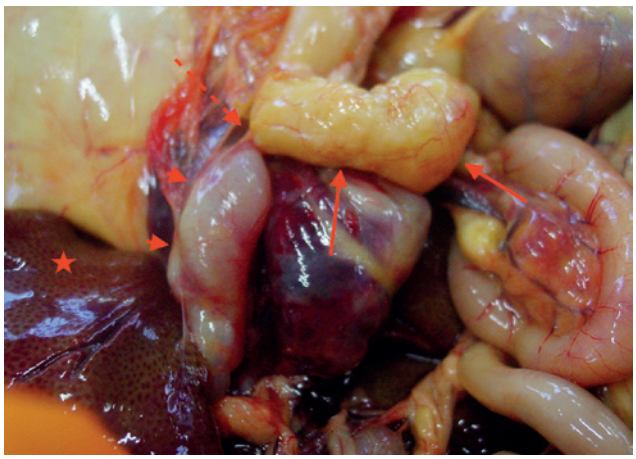


**Figure 4. Postmortem images at the time of necropsy. The duodenum is irregularly thickened (solid arrow) proximal to the entrance of the common bile duct (dashed arrow). The segmental dilation of the distal common bile duct close to the duodenal papilla is depicted (arrowhead). The yellow gross appearance of the surrounding mesenteric fat is explained by mild steatitis and saponification. The grossly visible zonal pattern of the liver is a normal finding in cats and might reflect fat storage (star).**

As morphological diagnosis, a duodenal adenocarcinoma with local metastasis to liver and lymph nodes and external compressive obstruction of the common bile- and pancreatic duct, causing jaundice and biliary fibrosis was stated.

## DISCUSSION

In the present case, the history, clinical presentation and the laboratory results match the reports previously published in cats with extrahepatic biliary obstruction (Head and Daniel, 2005; Mayhew et al., 2002; Buote et al., 2006). The main ultrasonographic finding leading to suspicion of extrahepatic biliary obstruction is a common bile duct diameter of > 5 mm, reported in 97 % of cats with extrahepatic biliary obstruction and intra hepatic dilatation of the bile duct (Gaillot et al., 2007). In a recent report, feline segmental dilations or choledochal cysts of the common bile duct have been described in four cats with chronic gastrointestinal clinical signs (Spain et al., 2017). Although the etiology of choledochal cysts is most likely multifactorial, it is unclear. Congenital and acquired etiologies have been reported (Grand et al., 2010). An abnormal pancreaticobiliary junction is the most common formulated hypothesis for the development of acquired choledochal cysts in human medicine (Stringer, 2018). The distinction of a choledochal cyst from an extrahepatic biliary obstruction is challenging and can be even more difficult with comorbid conditions, like in the present case (Spain et al., 2017). In this case, the common bile duct demonstrated a segmental dilatation proximal to the duodenal papilla and presented otherwise within normal limits. It is possible that a choledochal cyst developed secondary to the papillary neoplastic infiltration. However, a primary choledochal cyst cannot be excluded. Focal thickening of the intestinal wall can be caused by neoplastic and non-neoplastic disorders. Inflammatory changes usually lead to transmural thickening with preserved wall layering. However, in some instances, the wall layering can be altered (Gaschen, 2011). Intestinal neoplasia and infectious diseases often cause intestinal masses with loss of normal wall layering (Gaschen, 2011). The ultrasonographic differential diagnoses of duodenal neoplastic, infectious or inflammatory infiltration as underlying cause in the present case were based on those assumptions.

In human medicine, the most common cause of impaired bile excretion is choledocholithiasis and pancreatic carcinoma (Materne et al., 2000); diagnostics are based on sonographic, endosonographic, intraductal sonographic techniques and cross-sectional imaging modalities, like magnetic resonance tomography (MRT), computed tomography (CT) and PET-CT (Materne et al., 2000; Bakaeen et al., 2000). Endoscopic retrograde cholangiopancreatography has been established in human medicine to identify the location and cause of obstruction. Endoscopic ultrasonography is considered an alternative imaging method for determining extrahepatic cholestasis (Materne et al., 2000). MR tomography with MR cholangiopancreatography is a reliable procedure for the evaluation of the pancreas and the biliary system (Materne et al., 2000). In feline medicine, abdominal ultrasonography is the most commonly used diagnostic modality for imaging of the cholangiopancreatic area (Leveille et al., 1996; Gaillot et al., 2007) and was the main modality in the present case. Promising publications deal with the use of endosonography as diagnostic tool in veterinary medicine (Schweighauser et al., 2009). By positioning the endoscopic transducer within the lumen of the stomach or duodenum, the general visualization of the pancreas and the surrounding tissues has been improved compared to traditional transcutaneous ultrasound (Schweighauser et al., 2009). In the recent literature, MRT and MR cholangiography also provide promising additional imaging information in patients, in which the pancreas cannot be fully visualized ultrasonographically (Marolf et al., 2012). The cat in the present case report was euthanized due to guarded prognosis and financial concerns before reaching final diagnosis. Endoscopic, endosonographic or surgical exploration would potentially have provided more information and should be considered in similar cases. The potential palliative treatment options would have been of surgical or conservative nature depending on the degree of obstruction. Surgical considerations included biliary-enteric anastomosis or a biliary stent in case of complete obstruction (Mayhew et al., 2002). Duodenal adenocarcinoma was identified in this presented case after gross and histopathologic examination as underlying cause of the development of biliary obstruction. In different reports, feline intestinal adenocarcinoma makes up 0.4 % - 3.0 % of all feline tumors and 7 % - 27 % of all alimentary tumors (Kosovsky et al., 1988; Cribb et al., 1988; Mayhew et al., 2002; Gaillot et al., 2007; Gaschen et al., 2011; Rissetto et al., 2011). In 20 % - 61.5 % of cases, adenocarcinoma occurs in the small intestines compared to the large intestines (Rissetto et al., 2011). During postmortem examination in the present case, metastasis of the adenocarcinoma to the regional lymph nodes and the liver were identified. In accordance with the findings in the present case, metastatic spread of feline small intestinal adenocarcinoma has been seen in approximately 75 % of the cases into regional lymphnodes, liver and spleen (Kosovsky et al., 1988; Cribb et al., 1988).

In human medicine, primary duodenal adenocarcinoma is also a rare condition, representing approximately 0.3 % of all gastrointestinal tract carcinomas (Spira et al., 1977). Peripapillary tumors (duodenum, distal bile duct) are associated with a worse prognosis than primary tumors of the major duodenal papilla (Kim et al., 2001). Peripapillary tumors represent more than half of the duodenal adenocarcinomas (Bakaeen et al., 2000), and clinical obstructive jaundice is a common complication associated with

primary duodenal adenocarcinoma with an incidence ranging from 18 % to 54 % (Hung et al., 2007). Obstructive jaundice is considered a poor prognostic factor for patients with primary duodenal adenocarcinoma (Hung et al., 2007).

In conclusion, duodenal peripapillary adenocarcinoma should be considered as a rare, but potential cause of extrahepatic biliary obstruction in cats. With ultrasonography, it was possible to satisfactorily detect the location and cause of the obstruction. However, the differentiation between a papillary and a peripapillary origin remains challenging. With endosonography, the visualization would have been improved and would possibly have provided more information before euthanasia and so, more specific treatment options could have been discussed.

## REFERENCES


Bakaeen FG, Murr MM, Sarr MG, Thompson GB, Farnell MB, Nagorney DM, Farley DR, Van Heerden JA, Wiersema LM, Schleck CD, Donohue JH. (2000). What prognostic factors are important in duodenal adenocarcinoma? *The Archives of Surgery 135,* 635-642.

Barsanti JA, Higgins RJ, Spano JS, Jones BD. (1976). Adenocarcinoma of the extrahepatic bile duct in a cat. *Journal of Small Animal Practice 17,* 599-605.

Bressler EL, Rubin JM, McCracken S. (1987). Sonographic parallel channel sign: a reappraisal. *Radiology 164,* 343-346.

Brioschi V, Rousset N, Ladlow JF. (2014). Imaging diagnosis-extrahepatic biliary tract obstruction secondary to a biliary foreign body in a cat. *Veterinary Radiology & Ultrasound 55,* 628-631.

Buote NJ, Mitchell SL, Penninck D, Freeman LM, Webster CRL. (2006). Cholecystoenterostomy for treatment of extrahepatic biliary tract obstruction in cats: 22 cases (1994-2003). *Journal of the American Veterinary Medical Association 228,* 1376-1382.

Cribb A. (1988). Feline gastrointestinal adenocarcinoma: A review and retrospective study. *The Canadian Veterinary Journal 29,* 709-712.

D´Anjou MA, Penninck D. (2015). Liver. In: Penninck D and D´Anjou MA. (editors). *Atlas of Small Animal Ultrasonography*. Second edition, John Wiley & Sons, Inc., Iowa, p. 183-238.

Della Santa D, Schweighauser A, Forterre F, Lang J. (2007). Imaging diagnosis-extrahepatic biliary tract obstruction secondary to a duodenal foreign body in a cat. *Veterinary Radiology & Ultrasound 48,* 448-450.

Gaillot HA, Penninck DG, Webster CRL, Crawford S. (2007). Ultrasonographic features of extrahepatic biliary obstruction in 30 cats. *Veterinary Radiology & Ultrasound 48,* 439-447.

Gaschen L. (2011). Ultrasonography of small intestinal inflammatory and neoplastic diseases in dogs and cats. *Veterinary Clinics: Small Animal Practice 41*, 329-344.

Grand JG, Doucet M, Albaric O, Bureau S. (2018). Cyst of the common bile duct in a cat. *Australian Veterinary Journal 88,* 268-271.

Haines VL, Brown PR, Hruban RH, Huso DL. (1996). Adenocarcinoma of the hepatopancreatic ampulla in a domestic cat. *Veterinary Pathology 33*, 439-441.

Head LL, Daniel GB. (2005). Correlation between hepatobiliary scintigraphy and surgery or postmortem examination findings in dogs and cats with extrahepatic biliary obstruction, partial obstruction, or patency of the biliary system: 18 cases (1995-2004). *Journal of the American Veterinary Medical Association 227*, 1618-1624.

Hung FC, Kuo CM, Chuah SK, Kuo CH, Chen YS, Lu SN, Chien CSC. (2007). Clinical analysis of primary duodenal adenocarcinoma: an 11-year experience. *Journal of Gastroenterology and Hepatology 22*, 724-728.

Kelly DF, Baggott DG, Gaskell CJ. (1975). Jaundice in the cat associated with inflammation of the biliary tract and pancreas. *Journal of Small Animal Practice 16*, 163-172.

Kim MHK, Lee SK, Seo DW, Won SY, Lee SS, Min YI. (2001). Tumors of the major duodenal papilla. *Gastrointestinal Endoscopy 54,* 609-620.

Kosovsky JE, Matthiesen DT, Patnaik AK. (1988). Small intestinal adenocarcinoma in cats: 32 cases (1978-1985). *Journal of the American Veterinary Medical Association 192,* 233-235.

Lawrence D, Bellah JR, Meyer DJ, Roth L. (1992). Temporary bile diversion in cats with experimental extrahepatic bile duct obstruction. *Veterinary Surgery 21,* 446-451.

Leveille R, Biller DS, Shiroma JT. (1996). Sonographic evaluation of the common bile duct in cats. *Journal of Veterinary Internal Medicine 10,* 296-299.

Materne R, Van Beers BE, Gigot JF, Jamart J, Geubel A, Pringot J, Deprez P. (2000). Extrahepatic Biliary Obstruction: Magnetic Resonance Imaging Compared with Endoscopic Ultrasonography. *Endoscopy 32*, 3-9.

Mayhew PD, Holt DE, McLear RC, Washabau RJ. (2002). Pathogenesis and outcome of extrahepatic biliary obstruction in cats. *Journal of Small Animal Practice 43*, 247-253.

Mayhew PD, Weisse CW. (2008). Treatment of pancreatitis-associated extrahepatic biliary tract obstruction by choledochal stenting in seven cats. *Journal of Small Animal Practice 49*, 133-138.

Naus MJA, Jones BR. (1978). Cholelithiasis and choledocholithiasis in a cat. *New Zealand Veterinary Journal 26*, 160-161.

Neer TM. (1992). A review of disorders of the gallbladder and extrahepatic biliary tract in the dog and cat. *Journal of Veterinary Internal Medicine 6*, 186-192.

Schweighauser A, Gaschen F, Steiner J, Allenspach K, Francey T, Gaschen L. (2009). Evaluation of endosonography as a new diagnostic tool for feline pancreatitis. *Journal of Feline Medicine and Surgery 11*, 492-498.

Stringer M.D. (2018). Choledochal cysts. In: Barreto S., Windsor J. (editors). *Surgical Diseases of the Pancreas and Biliary Tree*. Springer, Singapore, p. 121-147.


**Vacature Dierenarts**

Waardeer jij ook deze mooie landelijke omgeving, dichtbij de mooie stad Groningen, waar de huizenprijzen zeer aantrekkelijk zijn en we geen last hebben van files?

***Dan zijn wij op zoek naar jou!***

***Wij zijn:*** Een maatschap gezelschapsdierenpraktijk in Winschoten, met een vast team van 3 dierenartsen en 6 assistentes.
Winschoten ligt in noordoost Nederland.

***Wij zoeken:*** Een enthousiaste collega dierenarts.
Ervaring niet vereist.

***Wij vragen:*** Iemand voor minimaal 3 tot 5 dagen per week,
die meedraait in het rooster van de weekdiensten (1x p/wk) en weekenddiensten. (1x p/5wk)
In bezit is van rijbewijs B.

***Wij bieden:*** De mogelijkheid je verder te specialiseren in bijvoorbeeld de echografie, endoscopie en de laparoscopische operatietechniek.
Salaris volgens cao.
Tijdelijke huisvesting is beschikbaar.

Reactie binnen 14 dagen richten aan:
DAPOG, Industrieweg 23, 9672 AP Winschoten
Of per email: administratie@dapog.nl

DAPOG DIERENARTSENPRAKTIJK OOST GRONINGEN

Dierenartsen Praktijk Oost Groningen

# Rare presentation of a squamous cell carcinoma originating from gastric compartment 1 in an alpaca

*Bijzondere presentatie van een squameus celcarcinoom afkomstig van compartiment 1 van de maag bij een alpaca*

**[1]K. Rosiers, [1]M. Strubbe, [2]T. Flahou, [1]H. Versnaeyen, [3]R. Ducatelle, [1]S. Roels**

[1]Dierengezondheidszorg Vlaanderen, Industrielaan 29, B-8820 Torhout
[2]Veterinary Practice for Alpacas, Dadizelestraat 32, B-8940 Geluwe
[3]Department of Pathology, Bacteriology and Poultry Diseases, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke

katrijn.rosiers@dgz.be

## ABSTRACT

**A twenty-year-old, male, intact alpaca with a history of anorexia, progressive weakness and recumbency was euthanized because of a poor prognosis and clinical deterioration. The animal was submitted for necropsy at the pathology department of Dierengezondheidszorg Vlaanderen (DGZ) diagnostic lab. A full necropsy was performed and showed a large firm white mass measuring 18x8x10 cm attached to the dorsal wall of gastric compartment 1 (C1) and expanding to the abdominal aorta. Miliary small white metastatic nodules were present on the pleura and peritoneum (carcinomatosis). Multiple small white metastatic nodules were also present in the parenchyma of the liver. On histological investigation, this neoplasia was characterized as squamous cell carcinoma (SCC).**

## SAMENVATTING

Een twintig jaar oude alpacahengst met een anamnese van anorexie, progressieve zwakte en frequenter neerliggen werd door de behandelende dierenarts geëuthanaseerd wegens een slechte prognose en een algemene klinische achteruitgang. Het dier werd aangeboden bij de afdeling pathologie van het diagnostisch labo DGZ Vlaanderen (Dierengezondheidszorg Vlaanderen). Tijdens autopsie werd een grote witte harde massa van 18x8x10cm aangetoond, die vastgehecht was aan de dorsale wand van maagcompartiment 1 (C1) en uitbreidend tot aan de abdominale aorta. Talrijke metastasen onder de vorm van kleine witte nodules waren aanwezig op de pleura en peritoneum (carcinomatose). Ook in het leverparenchym waren er meerdere metastatische kleine witte nodules aanwezig. Op histopathologisch onderzoek werd deze neoplasie getypeerd als een squameus celcarcinoom.

## INTRODUCTION

Alpacas and llamas belong to the group of new world camelids. They are hardy animals that are able to adapt to a wide range of management situations (Smith,1989). Anatomically, the anterior digestive tract is the most exceptional feature in camelids. Although camelids are foregut fermenters, they are not true ruminants as they lack the four well-defined stomachs of the ruminants. The anatomy of their three chambered stomach is markedly different from that of ruminants. Unlike ruminants, all of the compartments have glandular regions and none have papillae. C-1 fills the left abdomen and is divided into cranial and caudal sacs by a transverse pillar. The majority is non-glandular and is lined by unkeratinized stratified squamous epithelium (De la Vega, 1952; Luciano et al., 1980) but the ventral surfaces contain smaller glandular saccules lined by a mucinous glandular epithelium. C-2 has a glandular mucosal surface over the

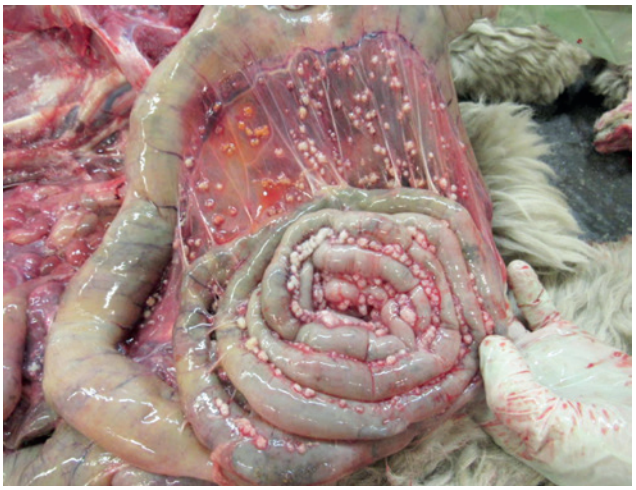


**Figure 1. Gross aspect of the mesentery and serosal surface of the intestine with multiple large and small metastatic nodules.**

greater curvature, which is subdivided by intersecting crests that create a retiform pattern not analogous to the pattern seen in the reticulum of ruminants. The lesser curvature shows a small area called the esophageal or ventricular groove (Vallenas, 1970). The primary crest margins are covered by stratified squamous epithelium extending from the ventricular groove, the secondary crests are covered with glandular mucosa (Vallenas, 1970). C-3 is lined entirely by glandular epithelium and secretes mucus and digestive enzymes (Vallenas et al., 1971; Cornick, 1988). The proximal four-fifths consists of a mucinous glandular epithelium similar to the saccules in C-1 and C-2 (PH 6.5). The terminal one-fifth of C3 is lined by true gastric glands, which secrete digestive enzymes and acid (PH <2) (von Engelhardt et al., 1979). Neoplasia is not common in camelids, with relatively few reports in the literature (Smith, 1989; Fowler, 2010). However, in a review by Valentine et al. (2007) on the prevalence of neoplasia in llamas and alpacas submitted for necropsy at a diagnostic laboratory, the prevalence in llama's was 11% and in alpaca's 4.9%. In that study, a difference in prevalence of neoplasia, tumor types and age at diagnosis between llamas and alpacas was also indicated (Valentine et al., 2007). Lymphosarcoma is the most commonly reported tumor in llamas and alpacas (Cebra et al., 1995; Irwin, 2001; Underwood et al., 1993; Sartin et al., 2004; Shapiro et al., 2005; Valentine et al., 2007; Rosa et al., 2013). Gastric squamous cell carcinoma has been previously reported in various animal species including a few cases in llamas (Cornick, 1988; Sartin et al., 1997; Valentine et al., 2007). However, to the authors' knowledge, no reports on the occurrence in alpacas have been published.

Clinical symptoms are non-specific and consist of weight loss, anorexia, vomiting and lethargy (Patnaik et al., 1980; Wester et al., 1980; Mc Kenzie et al., 1997). Squamous cell carcinoma of the forestomachs has been reported in the bovine rumen and ovine omasum and reticulum but are extremely rare (Doige, 1983; Bertone et al., 1985). Bovine ruminal squamous cell carcinoma is associated with consumption of bracken fern and could be the result of a mutagenic effect caused by interaction of the ptaquiloside carcinogen of bracken fern and bovine papillomavirus (Bertone et al., 1985).

In the present study, a rare presentation of a primary squamous cell carcinoma is reported in a twenty-year-old alpaca originating from gastric compartment 1 without evidence of mucosal lesions but expanding from the surface of the dorsal wall of C1 to the abdominal aorta and metastasizing to the liver and mesentery.

## Case history and necropsy

A twenty-year-old male intact alpaca was presented to the attending veterinarian with chronic weight loss, anorexia for already five days and an increased frequency of recumbency during the past fourteen days. The animal was kept for breeding purposes until the age of sixteen. He was born in Australia and subsequently stayed in New-Zeeland, England, France and Belgium. He did well until symptoms started four weeks prior to presentation to the veterinarian. Because of the poor prognosis, the animal was euthanized. The animal was submitted to the diagnostic laboratory DGZ Vlaanderen (Dierengezondheidszorg Vlaanderen), where a full necropsy was performed.

At necropsy, the animal was found to be thin and weighed 48 kg (reference mean 55 kg) (Fowler, 2010). The lower incisors were missing. The abdominal cavity contained a striking amount of clear yellow serous fluid (approximately 1200 ml). The mediastinal lymph nodes were enlarged, white and firm on cut section. The pleura was covered with multiple superficial small, white, well-circumscribed, slightly raised areas measuring approximately 0.3 cm. The coronary grooves of the heart showed cachectic serous fat atrophy. The mesentery and serosal surface of multiple abdominal organs showed multiple disseminated large and small (diameter range: 0.2-1cm), white nodules (Figure 1). One large (18x8x10cm), white, firm mass was attached to the first gastric compartment (C1) and expanded up to the abdominal aorta. The three gastric compartments contained food and there were no specific changes visible on the mucosal surface. The liver was enlarged and showed multifocal white soft nodules (average diameter 2cm) within the parenchyma.

## Histopathology and immunohistochemistry

Samples of the primary mass on C1 and thoracic and peritoneal nodules as well as liver and lung were fixed in 4%-neutral buffered formalin solution, routinely processed and embedded in paraffin. Five-µm-thick sections were mounted and stained with hematoxylin and eosin for histological examination.

Histopathologic evaluation of the mass of C1 re-

vealed a non-encapsulated and densely cellular neoplasm consisting of nests, anastomosing cords and islands of neoplastic cells, which were separated by a moderate amount of fibrovascular stroma with multifocal small numbers of lymphocytes (Figure 2). Neoplastic cells were polygonal with abundant eosinophilic to amphophilic cytoplasm. The nuclei of neoplastic cells were variably sized, sometimes large, oval to irregular, hyperchromatic to vesicular. They often contained a prominent single nucleolus. Many nests and islands of neoplastic cells show areas of necrosis characterized by karyorrhexis, karyolysis and pyknotic cell debris admixed with small numbers of neutrophils. Some neoplastic cells were undergoing disorderly individual cell keratinization (dyskeratosis). Multifocally, neoplastic cells surrounded variably-sized accumulations of concentric, lamellated eosinophilic material (keratin pearls). There were 2-5 mitoses per high power field with frequent bizarre mitotic figures. Anisokaryosis and anisocytosis were prominent. Multiple lymphatic vessels were dilated with intraluminal clusters of neoplastic cells attached to the lymphatic wall (tumor emboli).

Samples of the liver and mesenteric nodules showed a neoplastic process with similar histopathological characteristics as described for the primary mass.

Histopathologic examination of the lung showed multiple subpleural aggregates of cholesterol crystals surrounded by a rim of macrophages and multinucleated giant cells (cholesterol granulomas). No neoplastic lesions were present in the lung or on the pleura.

Immunohistochemical stains were performed on samples of the primary mass, liver and mesenterial metastases. In all tissue samples, neoplastic cells were positive for cytokeratin (monoclonal mouse anti-human Cytokeratin clone AE1/AE3, M3515, Agilent, Santa Clara, Unites States) and negative for vimentin (monoclonal mouse anti-Vimentin clone Vim 3B4, M7020, Agilent, Santa Clara, United States). Claudin staining (polyclonal rabbit anti-Claudin 1, 18-7362, Zymed Laboratories, South San Fransisco, United States) was strongly positive.

## DISCUSSION

Gastric squamous cell carcinomas generally present as exophytic vegetative ulcerated masses bulging into the lumen of the stomach (Munday et al., 2017). The behavior of the SCC in this alpaca was unusual because the tumor did not present as a mucosal nodular exophytic growth but expanded into the peritoneal cavity without causing any changes on the mucosal surface. A similar case without mucosal lesions has been reported in a man with a primary squamous cell carcinoma of the stomach presenting as a huge retroperitoneal tumor adjacent to the anterior wall of the abdominal aorta and invasive to the dorsal wall of the stomach (Wu et al., 2016). In that case however, there was no evidence of metastatic disease.

In human medicine, there are several hypotheses regarding the origin of SCC. According to one hypothesis, a metaplastic squamous focus is the origin; in another hypothesis, it has been suggested that SCC develops from an adenocarcinoma. Also growth of squamous cell tumors from undifferentiated stem cells mediated by unknown stimuli has been suggested (González-Sánchez et al., 2017). González-Sánchez et al. (2017) favor the theory that the SCC originates from foci of heterotopic squamous epithelium of the stomach submucosa or other layers. In human medicine, four histopathologic criteria for the diagnosis of squamous cell carcinoma have been described (Boswell and Helwig, 1965). These criteria of which at least one should be present to diagnose squamous cell carcinoma are 1. keratinized cell masses forming keratin pearls, 2. mosaic cell arrangement, 3. intercellular bridges and 4. high concentration of sulfhydryl and/or disulphide groups. However, the pathogenesis and etiology of SCC in the glandular stomach of human patients remain unclear (González-Sánchez et al., 2017).

The stomach is the most frequent location for gastrointestinal neoplasia with SCC reported most commonly in horses (Munday et al., 2017). Gastric SCC in horses develops in the proximal epithelial-lined portion of the stomach, and most commonly metastasizes transcoelomic after invading through the gastric serosa. There are also reports of lymphatic and hematogenous spread (Tennant et al., 1982; Taylor et al., 2009). Forestomach squamous cell carcinoma in ruminants is rare. In cattle, gastric SCC is often associated with esophageal papilloma caused by bovine papillomavirus 4 (BPV-4) and is specifically geographically spread (Kenya and North England) (Plowright, 1955; Bertone et al., 1985), being more common in certain regions of Scotland, England and Kenya (Sartin et al.,

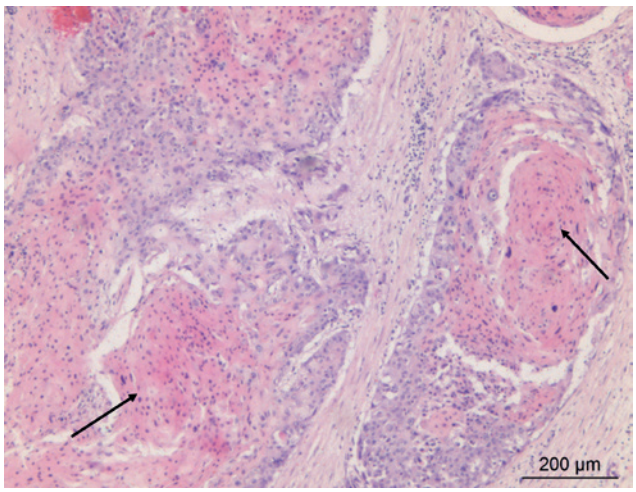



**Figure 2. Photomicrograph (hematoxylin eosin) of the primary tumor composed of nests and islands of neoplastic cells, separated by a moderate amount of fibrovascular stroma with multifocal small numbers of lymphocytes. Some nests of neoplastic cells present individual cell keratinization (arrows).**

1997). A mutagenic interaction between bovine papillomavirus and bracken fern ptaquiloside carcinogen can play a role in the development of gastric SCC in the rumen (Bertone et al., 1985). In a study by Sartin et al. (1997) on three llamas with gastric SCC, an environmental influence has been suggested because all three animals originated from the Southern United States. In some areas, where the genetic pool is rather narrow, genetic predisposition may be a factor to take into account (Sartin et al., 1997).

Although there are many similarities between the diseases of camelids and cattle, one should be cautious of extrapolating from ruminants to camelids (Esteban et al., 1988). Considering the differences in prevalence and type of neoplasia in llamas and alpacas, it is even questionable whether llamas and alpacas should be grouped and reported together. Neoplasia, such as lymphosarcoma, gastric SCC and adenocarcinoma are occasionally seen in these animals. However, due to their increasing popularity, pet status and related longevity, alpacas are more frequently presented to veterinary practices, which may increase the diagnosis of these neoplastic entities.

The clinical signs of anorexia, weight loss and lethargy present in the alpaca in the present case were similar to those reported in llamas and horses (Cornick, 1988; Sartin, 1997; Taylor et al., 2009). Routine blood analysis, gastroscopy, abdominocentesis and transabdominal ultrasound have been described as useful techniques for the diagnosis of gastric neoplasia in horses (Taylor et al., 2009). These diagnostic techniques are being implemented in well-equipped camelid practices and could be useful for the diagnosis of gastric neoplasms in camelids. Neutrophilia similar to that seen in horses with SCC has been reported in a llama (Cornick, 1988; Taylor et al., 2009). Given the location of the SCC in this case, the neoplastic mass would probably have been missed on gastroscopy. Nevertheless, abdominoscentesis, transabdominal ultrasound and blood analysis could have aided in diagnosing the SCC in this case.

Further research on the pathological conditions, predisposing factors and genetic predisposition is necessary to gain more thorough knowledge of the specific diseases and their diagnosis in these species.

## LITERATURE


Bertone A.L., Roth L., and O' Krepky J. (1985). Forestomach neoplasia in cattle: a report of eight cases. *Compendium on Continuing Education for the Practicing Veterinarian 7*, 85-90.

Boswell J.I., Helwig E. B. (1965). Squamous cell carcinoma and adenoacanthoma of the stomach. A clinicopathologic study. *Cancer 18*, 181-192.

Cebra C.K., Garry F.B., Powers B.E. (1995). Lymphosarcoma in 10 new world camelids. *Journal of Veterinary Internal Medicine 9(6),* 381-385.

Cornick J.L. (1988). Gastric squamous cell carcinoma and fasciolasis. *The Cornell Veterinarian 78*, 235-241.

De La Vega D.E. (1952). Histological aspects of the digestive system Alpaca. *Anales de Medicina de la Universidad Nacional Mayor de San Marcos, Lima 5,*163-187.

Doige C.E. (1983). Omasal squamous cell carcinoma in a ewe. *Canadian Journal of Comparative Medicine 47*, 382-384.

Engelhardt, W. von, Ali D.E., Wipper E. (1979). Absorption and secretion in the tubiform forestomach compartment three of the llama. *Journal of Comparative Physiology 132 (4)*, 337-42.

Esteban, L. R., Thompson, J. R. (1988). The digestive system of new world camelids - common digestive diseases of llamas. *Iowa State University Veterinarian 50*, 117-121.

Fowler M. E. (2010). Multisystemic disorders. In: Fowler M. E. (editor). *Medicine and Surgery of South American Camelids*. Third edition, Blackwell Publishing, Ltd, Ames, IA, Iowa, 271-288. .

Irwin J. A. (2001). Lymphosarcoma in an alpaca. *The Canadian Veterinary Journal 42,* 805-806.

González-Sánchez J.A., Vitón R., Collantes E., Rodríguez-Montes J.A. (2017). Primary squamous cell carcinoma of the stomach. *Clinical Medicine Insights*: *Oncology 11*, 1-4.

Luciano L., Reale E., von Engelhardt, W. (1980). The fine structure of the stomach mucosa of the llama Lama guanicoe. II. The fundic region of the hind stomach. *Cell and Tissue Research 208(2),* 207-228.

Mc Kenzie E.C., Mills J.N, Bolton J. R. (1997). Gastric squamous cell carcinoma in three horses. *Australian Veterinary Journal 75,* 480-483.

Munday J. S., Löhr C.V., Kiupel M. (2017). Tumors of the alimentary tract. In: *Tumors in Domestic Animals*. Fifth edition, Ames, Iowa John Wiley & Sons Inc. Stat Press, A Blackwell Publishing Company, 554-817.

Patnaik. A. K., Lieberman P. H. (1980). Gastric squamous cell carcinoma in a dog. *Veterinary Pathology 17,* 250-253.

Plowright W. (1955). Malignant neoplasia of the oesophagus and rumen of cattle in Kenya. *Journal of comparative Pathology 65*, 108-113.

Rosa F. B., Rissi D. R. (2013). Pathology in practice. Multicentric B-cell lymphoma. *Journal of American Veterinary Association 243,* 645-647.

Sartin E.A., Crowe D. R., Whitley E. M., Treat R. E., Purdy S. (2004). Malignant neoplasia in 4 alpaca's. *Journal of Veterinary Diagnostic Investigation 16,* 226-229.

Sartin E. A., Waldridge B. M., Carter DW, Herrera GA, Toivio-Kinnucan M., Lenz S.D., Pugh D. G., Wolfe D. F., Sundberg J.P. (1997). Gastric Squamous Cell Carcinoma in Three Llamas. *Journal of Veterinary Diagnostic Investigation 9*, 103-106.

Shapiro J. L., Watson P., McEwen B., Carman S. (2005). Highlights of camelid diagnoses from necropsy submissions to the animal Health Laboratory, University of Guelph, from 1998 to 2004. *Canadian Veterinary Journal 46,* 317-318.

Smith J. A. (1989). Noninfectious diseases, metabolic diseases, toxicities, and neoplastic diseases of south american camelids. *Veterinary Clinics of North America: Food Animal Practice 5 (1)*, 101-143.

Taylor S. D., Haldorson G. J., Vaughan B., Pusterla N. (2009). Gastric neoplasia in horses. *Journal of Veterinary Internal Medicine 23*, 1097-1102.

Tennant B., Keirn D. R., White K. K., Bentinck-Smith J., King J.M. (1982). Six cases of squamous cell carcinoma

of the stomach in the horse. *Equine Veterinary Journal 14*, 238-243.
Underwood J., Bell T. G., (1993). Multicentric lymphosarcoma in a llama. *Journal of veterinary diagnostic investigation 5,* 117-121.
Valentine B. A., Martin J. M. (2007). Prevalence of neoplasia in llamas and alpacas (Oregon State University, 2001-2006). *Journal of Veterinary Diagnostic Investigation 19*, 202-204.
Vallenas A., Cummings J. F., Munnell J. F. (1971). A gross study of the compartimentalized stomach of two new-world camelids, the llama and guanaco. *Journal of Morphology 134,* 399-423.
Vallenas P. A. (1970). Structural and functional studies of the llama and guanaco stomach. *Cornell University Dissertation and Abstract 31(6),*36-82.
Wester P. W., Franken P., Häni H. J. (1980). Squamous cell carcinoma of the equine stomach, A report of seven cases. *The Veterinary Quarterly 2,* 95-103.
Wu X. D., Zhou Y., Fan R. G., Zhou B., Shi Q., Jia J. (2016). Primary squamous cell carcinoma of the stomach presenting as a huge retroperitoneal tumor: case report. *Revista Espagnola de Enfermedades Digestivas 108* (*5),* 283-284.

**Uit het verleden**


Op onze Noordzeekust aangespoelde walvissen vormen nog steeds een sensatie. Vroeger werd er soms een exemplaar uitwendig geconserveerd en 'heelhuids' op een treinstel doorheen het hele land gevoerd. In stations van kleine en grote steden werd het reuzenbeest dan op een zijspoor enkele dagen tentoongesteld. Het terrein werd afgeschermd en het publiek kon binnen tegen betaling. Bij ons gebeurde dat nog in de jaren 1950. In recente jaren wordt autopsie uitgevoerd en van sommige exemplaren wordt het skelet geprepareerd en gemonteerd. Dat gebeurde in 2017 met de op de boeg van een schip in de Gentse haven gearriveerde Blauwe Vinvis die na conservering in de Sint-Baafskathedraal werd opgehangen. Het bijbelverhaal 'Jonas en de walvis' werd daarvoor gebruikt als excuus. Nu kan je 'Leo', zoals hij genoemd wordt, bewonderen en bestuderen boven in het restaurant van de Campus Diergeneeskunde Merelbeke.

Luc Devriese

# Infectious canine hepatitis, not only in the textbooks: a brief review and three case reports

*Infectieuze caniene hepatitis, niet alleen in de handboeken: een beknopt overzicht en drie gevalstudies*

**B. De Jonge, L. Van Brantegem, K. Chiers**

Department of Veterinary Pathology, Bacteriology and Poultry Diseases, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium

bert.dejonge@ugent.be

## ABSTRACT

**Infectious canine hepatitis (IHC) caused by canine adenovirus 1 (CAdV-1) is a classic disease in dogs causing severe illness in non-vaccinated dogs. For this reason, CAdV-1 is incorporated in the standard core vaccination. Because of widespread vaccination, this disease is only rarely seen by the veterinary practitioner. However, ICH does occur in Belgium, especially when vaccination is not adequate. In this brief review, the authors intend to refresh the knowledge of ICH illustrated by three recent cases.**

## SAMENVATTING

Infectieuze caniene hepatitis (IHC) veroorzaakt door het caniene adenovirus 1 (CAdV-1) is een klassieke ziekte bij honden die een ernstig verloop kent bij niet-gevaccineerde honden. Om deze reden is CAdV-1 opgenomen in de standaardvaccinatie. Door de wijdverspreide vaccinatie wordt deze ziekte nog slechts zelden gezien door de dierenarts. ICH komt echter wel voor in België, vooral wanneer de vaccinatie niet adequaat is. Met deze beknopte review willen de auteurs de kennis van ICH opfrissen, geïllustreerd met drie recente gevallen.

## ETIOLOGY

Canine adenovirus 1 (CAdV-1) causes infectious canine hepatitis (ICH), also called epizootic fox encephalitis or Rubarth's disease. Infection can occur in dogs but also wild *Canidae* (e.g. foxes, wolves and coyotes), *Ursidae* (bears) and *Mustelidae* (e.g. martens, weasels, sloats) can be infected (Greene, 2012; Dowgier et al., 2018). Viral shedding occurs through all secretions and excretions in the acute stage. Up to nine months post infection, the virus can persist in the kidneys with presence of the virus in the urine (Baker et al., 1954; Willis, 2000). Adenoviruses are very resistant in the environment, surviving several days at room temperature and several months at ≤4°C. Canine adenovirus 2 (CAdV-2) causes mild self-limiting infection of the upper respiratory tract and is a contributor to infectious tracheobronchitis (ITB) known as kennel cough (Greene, 2012; Day et al., 2020).

## PATHOGENESIS

After oronasal exposure, viral replication causes an often severe tonsillitis and pharyngitis (Cullen and Stalker, 2016). The virus translocates in the bloodstream causing viremia and reaching its primary targets: endothelial cells and hepatocytes (William, 2000). The main organs affected by CAdV-1 are liver, kidneys and eyes (Greene, 2012). Hepatocellular viral replication causes widespread necrosis. A sufficient antibody response clears the virus and restricts the hepatic damage with complete regeneration. When a partial neutralizing antibody titer is present at 4 to 5 days post infection (dpi), persistent hepatic inflammation continues and chronic active hepatitis develops finally resulting in hepatic fibrosis. A persistently low antibody titer will result in extensive hepatic necrosis and death (Greene, 2012).

Throughout the body, the virus targets the vascular endothelial cells. In the kidneys, viral replication pri-

mary injures the capillary network of the glomerulus. Because of immune complex formation, glomerulonephritis develops with transient proteinuria (Decaro et al., 2008; Greene, 2012). In recovered dogs, the virus persists in the renal tubular epithelium resulting in urinary viral shedding for several months (Baker et al., 1954; Willis, 2000; Greene, 2012). A mild non-progressing interstitial nephritis may be present after recovery (Cullen and Stalker, 2016).

Ocular lesions develop in approximately 20% of dogs after recovering from natural disease. In a first stage, viral replication occurs in the endothelium of the anterior uvea (the iris) and the inner side of the cornea. This results in mild uveitis with often photophobia during clinical or subclinical disease (Willis, 2000). In a later stage, more severe keratouveitis with corneal edema develops. After seroconversion (at 6-7 dpi), immune complexes (type III hypersensitivity) damage the corneal endothelium causing corneal edema (‘blue eye’) (Wilcock and Njaa, 2016). No viral replication is present at this stage (Willis, 2000). In severe cases, blocking of the iridocorneal filtration angle can cause glaucoma (Greene, 2012).

A common complication of the acute disease is diffuse intravascular coagulation (DIC). DIC develops because of widespread endothelial damage with systemic intravascular blood clotting and secondary consumption of the coagulation factors and thrombocytes. Because of systemic endothelial damage and DIC, generalized petechial and ecchymotic bleedings and edema can develop throughout the body (Greene, 2012; Cullen and Stalker, 2016).

## CLINICAL FINDINGS

ICH occurs most common in dogs younger than one year but can occur in unvaccinated dogs of all ages (Green, 2012). The incubation period is two to five days (Cabasso, 1962). In the early phase, a biphasic fever (<40°C) is the first manifestation (Decaro et al., 2008). The clinical course is variable, disease can develop very acutely with death in a few hours after the onset of clinical signs (Cabasso, 1962). In a less acute course, common clinical signs are depression, loss of appetite, increased heart rate, hyperventilation, coughing, vomiting and diarrhea. Although the liver is often severely affected by the disease, icterus is only rarely seen. Tonsillitis and pharyngitis can be severe, cervical lymphadenopathy is frequently present. Because of endothelial damage and DIC, petechial and ecchymotic hemorrhages can occur on mucosae and skin (Decaro et al., 2008; Greene, 2012). Abdominal distention develops because of accumulation of serosanguineous fluid and hepatomegaly. Although rare, some dogs develop neurological signs (ataxia, seizures, coma), which is associated with damage and bleeding of small caliber vessels in the brain (Decaro et al., 2008; Cullen and Stalker, 2016). Neurological signs are more prevalent in foxes explaining the alternative disease name ‘fox encephalitis’ in these animals (Vandevelde et al., 2012). The uncomplicated disease course of ICH takes five to seven days before improvement (Greene, 2012). Keratouveitis with corneal opacity (‘blue eye’) may develop one to three weeks after recovery and results in photophobia, blepharospasm and ocular discharge (Wills, 2000; Decaro et al., 2008).

## PATHOLOGICAL FINDINGS

Post mortem lesions in spontaneously fatal acute cases are usually suggestive for a gross diagnosis of ICH. The liver is only slightly enlarged and is turgid and friable with sometimes congestion and mottling (Cullen and Stalker, 2016) (Figure 1A). The wall of the gall bladder is thickened due to edema, which is thought to be a pathognomonic feature (Vandevelde et al., 2012) (Figure 2). Lymph nodes are enlarged, edematous and hemorrhagic. Petechial and ecchymotic bleedings may be present on the serosal surfaces (Figure 3); there is usually a small amount of serosanguineous fluid in the abdomen. Gross lesions in other organs are variable and include mainly hemorrhage. Involved organs are classically brain, lungs, and the bones in younger dogs (Cullen and Stalker, 2016).

Histological lesions in the liver are centrilobular zonal necrosis with scattered deeply acidophilic intranuclear viral inclusion bodies in numerous hepatocytes and Kupffer’s cells (Figures 1B, 1C). Histological lesions in other organs are mainly the result of endothelial damage and DIC consisting of hemorrhage and edema (Cullen and Stalker, 2016). Viral inclusions can also be found in the renal glomeruli and rarely in the tubular epithelium. Focal interstitial nephritis is often present long after the infection (Cullen and Stalker, 2016). Lymph nodes and spleen are reactive and congested with mononuclear cell infiltrates and numerous lymphoid follicles with central necrosis (Greene, 2012).

During convalescence, ocular manifestation may develop, most frequently unilateral. There is diffuse clouding of the cornea (‘blue eye’) with a typical granular aspect due to interstitial edema; conjunctival inflammation is frequently apparent (Willis, 2000). Histological lesions are bilateral although usually of unequal intensity explaining the clinical unilateral manifestation. Histologically, an anterior uveitis is present with lymphocytes and plasma cells surrounding vessels in the iris and ciliary body. There is hydropic degeneration of the corneal endothelium with secondary stromal edema. An intranuclear viral inclusion can rarely be seen. Uncommon complications are interstitial keratitis with fibrosis, synechia posterior and angle obstruction (glaucoma) (Wilcock and Njaa, 2016).

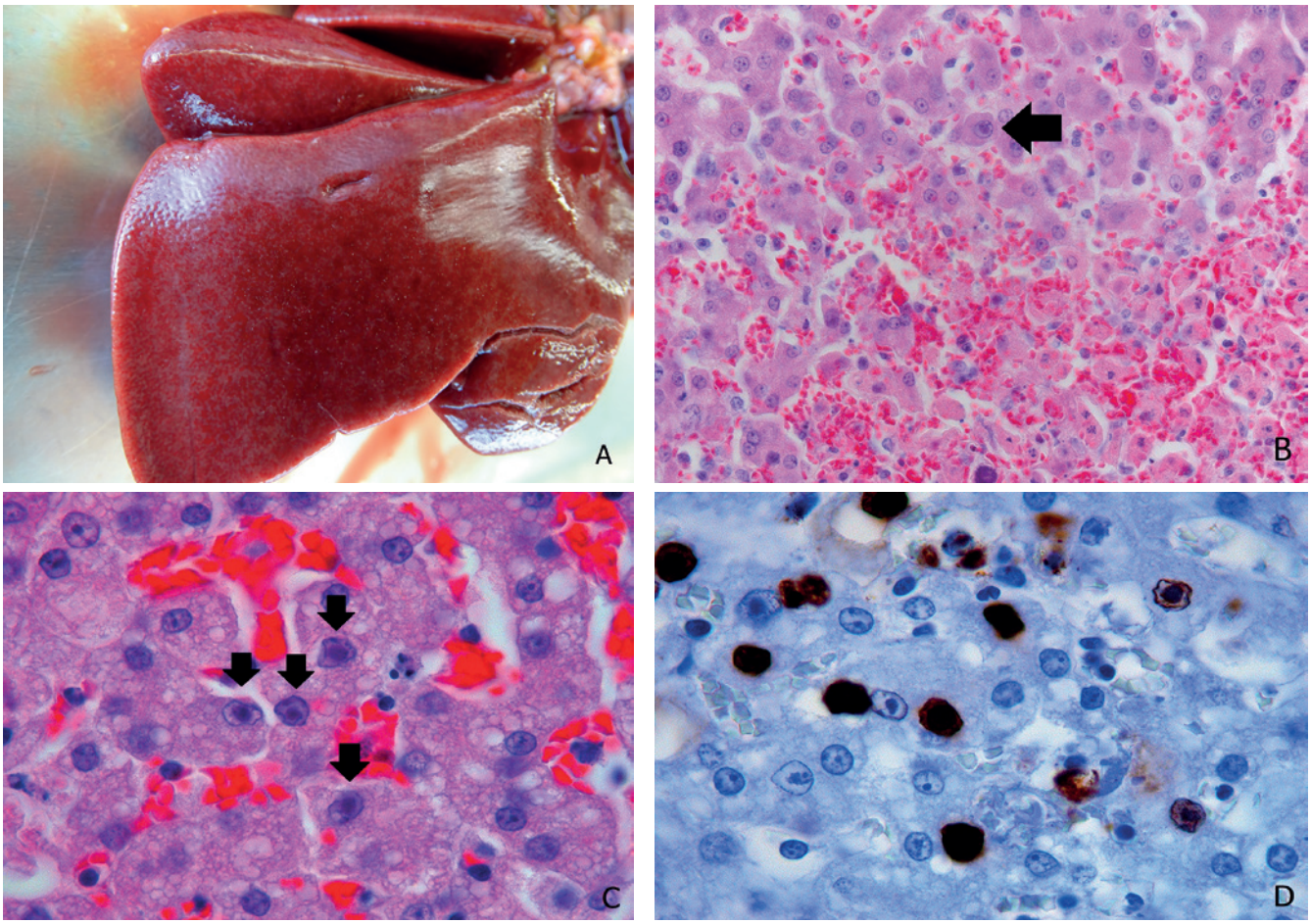



**Figure 1. Liver of the dogs in case 1 (A, B) and case 2 (C, D). A. The liver is turgid and friable causing it to rupture easily during post mortal examination. B. Grossly, there is moderate mottling of the parenchyma (A) histologically characterized by necrotizing hepatitis with large, eosinophilic, intranuclear viral inclusions surrounded by a clear halo and marginated chromatin are typical for adenovirus infection (arrows). In case 1, necrosis is prominent at the lower portion of the picture with disruption of hepatic cord architecture, cytoplasmic eosinophilia, karyorrhexis and pooling of blood. HE 400X. C. In case 2, diffuse hepatocytic vacuolar degeneration is characterized by hepatocyte swelling with cytoplasmic vacuolization and numerous inclusions (arrows). D. On immunohistochemistry, numerous hepatocytes and Kupffer's cells stain positive for CAdV, HE 1000X.**

## DIAGNOSIS, TREATMENT AND PREVENTION

In the early stage, blood examination reveals leukopenia, lymphopenia and neutropenia. Neutrophilia, lymphocytosis, increased alanine transaminase (ALT) and aspartate aminotransferase (AST) develop in a further course (Greene, 2012). Also coagulation abnormalities consistent with DIC are usually apparent during the viremic stage. Ante mortem confirmation is possible through serological testing. Post mortem gross and histological lesions are highly indicative; confirmation can be done by immunohistochemistry, immunofluorescence or polymerase chain reaction (PCR) (Greene, 2012).

Treatment of dogs with ICH is mainly supportive and comprises intravenous fluid therapy, treatment of the DIC, hypoglycemia and reducing endogenic ammonia production (hepatoencephalopathy) (Greene, 2012). Inactivation of the virus occurs at 50-60°C in five minutes, so washing or steaming of textiles at these temperatures is a possibility for disinfection. Chemical disinfection can be done by iodine, phenol and sodium hydroxide (Greene, 2012).

In countries where adequate vaccination is performed by cross-protective CAdV-2 modified live virus, ICH has effectively been controlled and has almost been eliminated from the domestic canine population (Dowgier et al., 2018). A recommended protocol is vaccination at eight to ten and twelve to fourteen weeks with three to four weeks in between. Clinical disease has never been reported in an adult dog with adequate vaccination in puppyhood, but booster vaccination every three years is indicated (Greene, 2012).

## CASE DESCRIPTIONS

### Case 1

A female Rhodesian Ridgeback of 15 weeks developed lethargia, ventroflexion and partial anorexia.

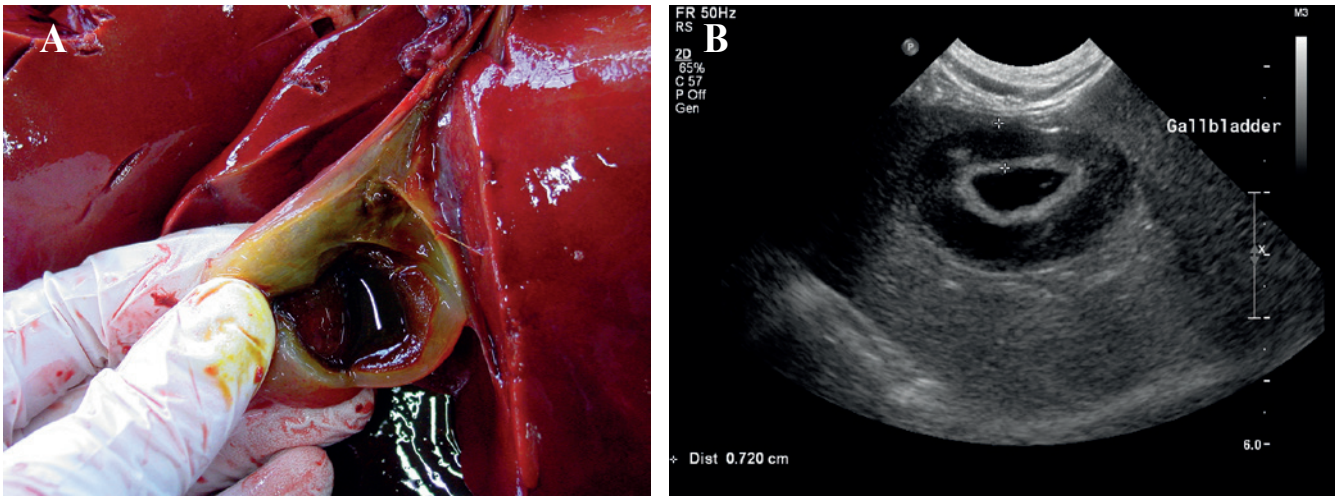



**Figure 2. Gall bladder of the dog in case 1. A. Gross examination and B. Transverse section on premortal ultrasound examination. There is marked thickening of the gall bladder wall due to edema. Gall bladder edema is thought to be a pathognomonic lesion of canine infectious hepatitis.**

Three days later, the dog was admitted for emergency because of development ataxia and head pressing. Blood examination revealed increased liver enzymes. On ultrasound examination, a generalized abdominal lymphadenopathy, mild hepatomegaly with severely thickened gall bladder and a small amount of peritoneal effusion were noticed (Figure 2). Blood examination revealed a coagulopathy with severe thrombocytopenia (23 K/µL; normal range: 148-484), upper limit prothrombin time (PT) (18s; normal range: 11-17) and markedly increased activated partial thromboplastin time (aPTT) (144s; normal range: 72-102). Finally, cardiac arrest developed, with unsuccessful reanimation. Serological testing for toxoplasmosis, neosporosis, parvovirosis and angiostrongylosis was negative. The clinicians postulated hemorrhagic disorder, infectious or immune mediated encephalitis and metabolic encephalopathy as main differential diagnoses. The owner was a breeder of Rhodesians. The pup belonged to a litter of eleven but only one other pup was present at the moment of the disease. Although they slept and lived together, this other pup did not develop any signs. Vaccination against canine distemper virus and canine parvovirus was performed at eight weeks, vaccination against *Bordetella bronchiseptica* and canine Parainfluenza virus at ten weeks. A rabies vaccine was administered at twelve weeks. The first core vaccination was planned at sixteen weeks of age.

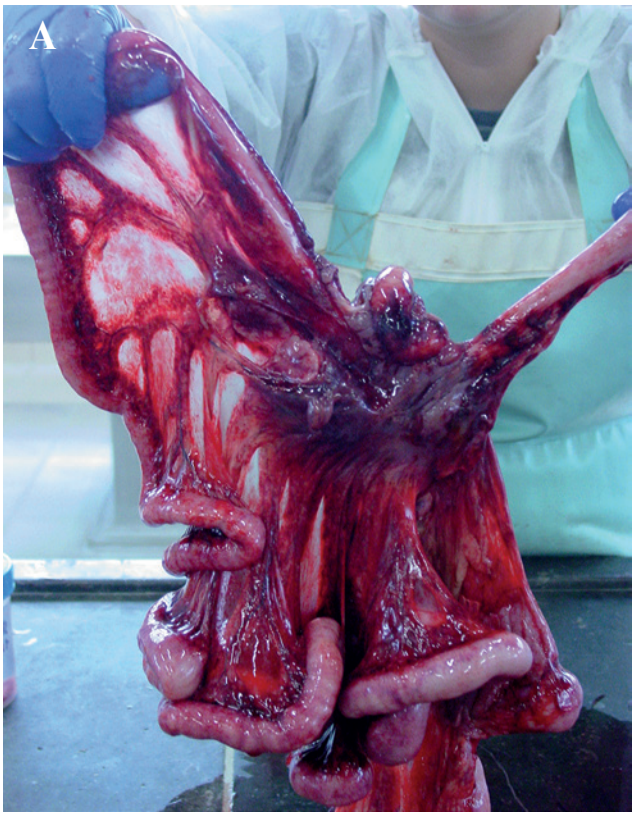
At necropsy, the liver was moderately pale, enlarged and mottled with a scant amount of fibrin strands on the capsule with severe edema of the gall bladder (Figures 1A and 2). There were several superficial ruptures secondary to reanimation causing hemoabdomen. The lymph nodes were severely enlarged and hemorrhagic, the spleen was moderately enlarged with white pulp hyperplasia at cut surface. The mesenteric lymph nodes were edematous and hemorrhagic on cut surface. Extensive petechial and ecchymotic hemorrhages covered the abdominal serosal surfaces (Figure 3). In the brain, dispersed small hemorrhages were bilaterally present in the grey and white matter of the basal nuclei and in the parenchyma of the thalamus (Figure 4).

Histologically, severe multifocal midzonal to centrilobular hepatic necrosis with large eosinophilic intranuclear viral inclusion bodies was present (Figure 1B). The mesenteric lymph nodes were highly reactive with proliferation of lymphoblasts and secondary follicle formation. In the brain, there was presence of disseminated perivascular hemorrhage, most extensive in the basal ganglia and thalamus (Figure 4). Immunohistochemical staining for CAdV revealed numerous positive staining hepatocyten and Kupffer's cells in the liver, fewer positive staining macrophages in the mesenterial lymph node and renal glomeruli (mesangial cells). Also in the brain, several affected bloodvessels showed positive staining endothelial cells.

## Case 2

A male Chihuahua of fourteen weeks died within two days after developing high fever with nasal discharge at a small dog breeding facility. Three other pups, one from the same and two from another litter, developed comparable clinical signs in the same week but recovered. The pups are home bread and do not have contact with other dogs than the owner's. The core vaccination is administered at ten weeks of age with repetition at fourteen weeks. The owner introduced a new adult male from Russia seven months prior to the disease manifestation; this dog never showed any clinical signs and was well-vaccinated. The pups are only allowed to go outside on a walled courtyard.

At necropsy, no gross lesions typical of ICH were present. Only an enlarged pale liver and spleen, focal



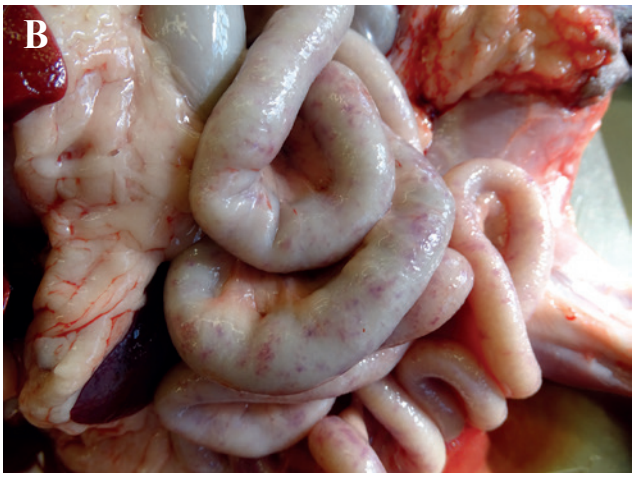




**Figure 3. Intestine and mesentery of the dogs in A. case 1 and B. case 3. Both pictures display intestinal serosal hemorrhage, although more extensive and suffusive within the mesentery and mesenteric root of the dog in case 1. Hemorrhagic diathesis is a common sequela of fulminant infection due to DIC and endothelial damage.**

renal hemorrhage and melena were the most obvious findings.

At histological examination, there was panlobular vacuolar degeneration of hepatocytes with numerous intranuclear acidophilic inclusions (Figure 1C). Identical inclusions were present in the spleen and kidneys within respectively, macrophages and glomerulic mesangial or endothelial cells. The spleen is highly reactive, displaying hyperplasia of the white pulp with often central necrosis of lymphoid follicles. Immunohistochemical staining of the liver for CAdV highlights numerous positive staining nuclei and fewer positive glomerular mesangial cells in the kidney (Figure 1D).

### Case 3

A female Staffordshire Bull Terrier of twelve weeks old developed high fever (40°C) and lethargy. At ultrasound examination, a thickened gall bladder wall with an anechogenic enlarged liver and free abdominal fluid was noticed. The pup died between 24 and 36 hours after hospitalization. The dog had been in the owner's possession for three weeks after buying it at a small breeding facility. One other well-vaccinated adult dog had been with the owner for years. The pup described in this case had only received the first core vaccination at eight weeks of age. A week before the illness, the dog of a friend entered the house and had contact with the pup. This dog did not display any signs of disease at that time.

At necropsy, the liver was moderately enlarged, turgid and friable with a mottled aspect and scattered fibrin deposition at the capsular surface. The gall bladder wall was severely thickened and edematous. There was generalized lymphadenopathy with marked edema and splenomegaly. The small intestine and urine bladder displayed moderate multifocal serosal hemorrhages (Figure 3). The abdominal cavity contained an abundant amount of serosanguineous fluid (200ml). The stomach and duodenum contained a moderate amount of dark brown mucous content (digested blood).

At histological examination of the liver, multifocal midzonal to centrilobular coagulation necrosis with numerous eosinophilic hepatocellular and endothelial intranuclear viral inclusion bodies were present. The spleen and mesenteric lymph node were highly reactive. Immunohistochemical staining for CAdV revealed numerous positive staining hepatocytes and Kupffer's cells in the liver, fewer positive staining macrophages in the mesenterial lymph node and renal glomeruli (mesangial cells). Also in the brain, several bloodvessels showed positive staining endothelial cells, although no histological lesions were present at hematoxylin-eosin staining.

## DISCUSSION

ICH is a classical disease in dogs, although it is very rarely encountered by most veterinary practitioners due to widespread vaccination (Balboni et al., 2014; Dowgier et al., 2018). Most veterinarians are not familiar with the clinical or pathological manifestations of this disease, although ICH does occur in Belgium. The patients in the three present cases were admitted

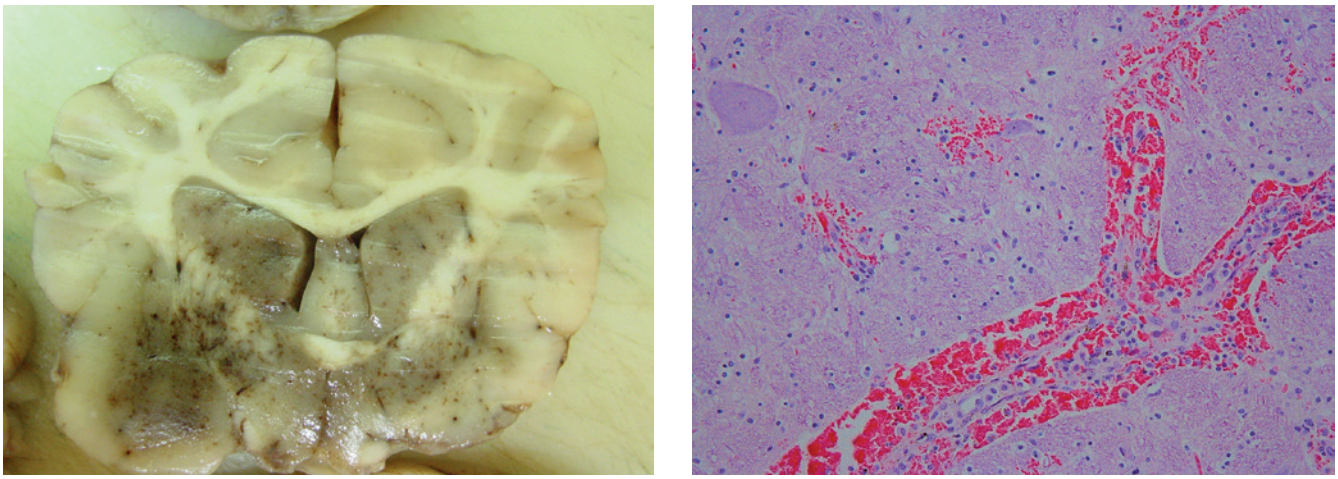


**Figure 4. Brain of the dog in case 1. Bilaterally dispersed small hemorrhages in the grey and white matter within the basal nuclei of the cerebrum, gross and histologically. Microvascular bleeding in the brain is a rare development in dogs but a common finding in wild species explaining the more pronounced neurological signs in foxes. In these animals, ICH is called fox encephalitis. HE 200X.**

in a period of two months at the necropsy facility of the Faculty of Veterinary Medicine (Ghent University) in 2019. This is remarkable because only four other non-co-occurring ICH cases have been seen at the necropsy facility since 2010; this in comparison to 743 cases of parvovirosis and a total of 3065 necropsied dogs in the same period. A common source of infection for these three cases was however not likely because of a scattered geographical distribution and different origin of the three pups. The source of infection for all three cases could not be traced. However, in one case (2), contact with a foreign adult dog could have been the cause. It was a Russian male dog, which had been introduced seven months earlier. As urinary excretion has been demonstrated in cases of ICH up to nine months post infection (Baker et al., 1954), it could be possible that the Russian dog was still infectious after being infected abroad. Unfortunately, except for correct vaccination according to the owner, no history of the Russian dog was available. In addition, the virus is quiet resistant in the environment increasing the importance of indirect transmission and making it more difficult to track down the source of the infection (Greene, 2012).

The fifteen-week-old Rhodesian Ridgeback in case 1 did not receive its first core vaccination, leaving it vulnerable for infection. The owner believed the combination of the rabies and core vaccination would be too much of a burden for the pup, being the reason for the delay of the core vaccination. Curiously, the only other remaining pup from the same litter was not vaccinated neither; it lived and slept together with the infected pup but did never develop any signs of disease. The fourteen-week-old Chihuahua in case 2 and the twelve-week-old Staffordshire Bull Terrier in case 3 received their first core vaccination but not the booster. A second vaccination is essential for adequate immunity explaining the susceptibility of these dogs once maternal immunity had disappeared (Greene, 2012). The dams in all three cases were well-vaccinated according to the owners. After the diagnosis had been made in case 2, the vaccination protocol was changed from ten and fourteen weeks to nine and twelve weeks. No other cases emerged in the following two litters in the next period of one year.

Another possible source of infection is wild carnivores susceptible to CAdV-1, in Western Europe being mainly foxes and mustelids. In some studies, a CAdV seroprevalence has been indicated in red foxes (*Vulpes vulpes*) of 19% to 64.4% in the UK (Thompson et al., 2010; Walker et al., 2016a), 3,5% in Germany (Truyen et al., 1998), and 59,6% in Scandinavia (Akerstedt et al., 2010). Unfortunately, the serological examinations cannot distinguish CAdV-1 from CAdV-2. However, CAdV 1 infection in foxes has been reported in the UK and Germany (Walker et al., 2016b; Verin et al., 2019 ). Red foxes are the most prevalent free-ranging species susceptible to ICH in Europe, and due to their intrusive behavior and high population density, they might play a major role in the disease epidemiology (Dowgier et al., 2018). Virus is excreted through urine and feces, so dogs might be attracted by the smell and infect themselves through oronasal contact with these excreta (Balboni et al., 2014). Also in Flanders, this species is common with occurrence in urban areas (Van Den Berge et al., 2013). Stone martens (*Martes foina*) belong to the *Mustelidae,* which are known to be sensitive to CAdV-1 (Greene, 2012). In Flanders, these animals have been recolonized during the past 25 years (Van Den Berge et al., 2012). Although no cases of ICH or seroprevalence in martens have been reported, they should be regarded as a possible source of infection for domestic dogs.

Neurological manifestation of ICH typically occurs in wild species (e.g. foxes) but are rarely encoun-

tered in domesticated dogs (Cabasso, 1962; Hornsey et al., 2019). Nevertheless, case 1 exhibits typical lesions for involvement of the central nervous system as is seen in fox encephalitis (Cabasso, 1962; Vandevelde et al., 2012). So, it is important to keep CAdV-1 in the differential diagnosis when confronted with a young unvaccinated dog with neurological signs.

The pathological lesions are variable in the described cases. At histological examination of the liver in case 2, there was diffuse vacuolar degeneration of hepatocytes with numerous viral inclusions and without the characteristic presence of hepatic necrosis. Most likely, this animal died in an early stage before hepatic necrosis could develop. This is supported by the fact that gall bladder edema was neither present in this case, nor were hemorrhages and lymphadenopathy. In experimental infection, viral inclusions can be histologically detected after three days, reaching its peak at day 4 and declining at day 5 and 6; at day 8 inclusions can only be found occasionally (Cabasso, 1962). This suggests this animal has been infected around four days prior to its death. Hence, it is important for the pathologist and the clinician to keep in mind acute cases might not exhibit the typical lesions. When a veterinarian is encountered with a dog suspected of ICH, it is useful to perform an ultrasound examination of the abdomen. Gall bladder edema in a young dog, especially in combination with fever, hepatomegaly and systemic lymphadenopathy, is diagnostic for ICH (Vandevelde et al., 2012). However, absence of gall bladder edema does not exclude ICH. In two out of the three cases, gall bladder edema was prominent and was noticed during ultrasound examination by the veterinarian.

In conclusion, in this paper, three cases of canine infectious hepatitis are reported in pups of 15, 14 and 12 weeks of age, respectively. As ICH might be a rare disease, it is not extinct, so adequate vaccination is still necessary because of subclinical carriers, environmental resistance of the virus and circulation in wild carnivores. Two out of the three cases manifested extensive gall bladder edema, which is in agreement with the fact gall bladder edema is typical of ICH. This might be useful for the establishment of a clinical diagnosis through ultrasound examination. It is also important to keep CAdV-1 in the differential diagnosis when confronted with a young, unvaccinated dog with neurological signs.

## REFERENCES


Akerstedt, J., Lillehaug, A., Larsen, I.L., Eide, N.E., Arnemo, J.M., Handeland, K., (2010). Serosurvey for canine distemper virus, canine adenovirus, Leptospira interrogans, and Toxoplasma gondii in free-ranging canids in Scandinavia and Svalbard. *Journal of Wildlife Diseases 46*, 474-480.

Baker I. A., Jensen H. E., Witter R. E. (1954). Canine infectious hepatitis - fox encephalitis. *Journal of the American Veterinary Medical Association 124*, 214-216.

Balboni A., Mollace C., Giunti M., Dondi F., Prosperi S., Battilani M. (2014). Investigation of the presence of canine adenovirus (CAdV) in owned dogs in Northern Italy. *Research in Veterinary Science 97*, 631-636.

Cabasso V.J. (1962). Infectious canine hepatitis virus. *Annals of the New York Academy of Sciences 101*, 498-514.

Cullen J.M., Stalker M.J. (2016). Liver and biliary system. In: M. G. Maxie (editor). *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*. Sixth edition, St. Louis MO, Elsevier Ltd, 310-312.

Day M.J., Carey S., Clercx C., Kohn B., Marsillo F., Thiry E., Freyburger L., Schulz B., Walker D.J. (2020). Aetiology of canine infectious respiratory disease complex and prevalence of its pathogens in europe. *Journal of Comparative Pathology 176*, 86-108.

Decaro N., Martella V., Buonavoglia C. (2008). Canine adenoviruses and herpesvirus. *Veterinary Clinics: Small Animal Practice 38*, 799-814.

Dowgier G., Lahoreau J., Lanave G., Losurdo M., Varello K., Lucente M.S., Ventriglia G., Bozzetta E., Martella V., Buonavoglia C., Decaro N. (2018). Sequential circulation of canine adenoviruses 1 and 2 in captive wild carnivores, France. *Veterinary Microbiology 221*, 67-73.

Green R.G., Ziegler N.R., Dewey E.T., Shillinger J.E. (1931). Epizootic fow encephalitis. III. Experimental transmission. *American Journal of Epidemiology 14*, 353-373.

Greene C.E. (2012). Infectious canine hepatitis and canine acidophil cell hepatitis. In: Greene C.E., (editor). *Infectious Diseases of the Dog and Cat*. Fourth edition, St Louis, MO. Saunders Elsevier, 42-47.

Hornsey S.J., Philibert H., Godson D.L., Snead E.C.R. (2019). Canine adenovirus type 1 causing neurological signs in a 5-week-old puppy. *BMC Veterinary Research 418*, 2-6.

Rubarth H.S. (1947). An acute virus disease with liver lesion in dogs (hepatitis contagiosa canis). A pathologico-anatomical and etiological investigation. *Acta Pathologica, Microbiologica, et Immunologica Scandinavica 69*, 222.

Thompson H., O'Keeffe A.M., Lewis J.C.M., Stocker L.R., Laurenson M.K., Philbey A.W. (2010). Infectious canine hepatitis in red foxes (Vulpes vulpes) in the United Kingdom. *Veterinary Record 166*, 111-114.

Truyen, U., Müller, T., Heidrich, R., Tackmann, K., Carmichael, L.E., (1998). Survey on viral pathogens in wild red foxes (Vulpes Vulpes) in Germany with emphasis on parvoviruses and analysis of a DNA sequence from a red fox parvovirus. *Epidemiology and Infection 121*, 433-440.

Van Den Berge K., Gouwy J., Berlengee F., Vansevenant D. (2012). Populatie-ontwikkeling van de steenmarter *Martes foina* in Vlaanderen in relatie tot schaderisico's. Rapporten van het Instituut voor Natuur- en Bosonderzoek 2012 (rapportnr. bv. 62). Instituut voor Natuur- en Bosonderzoek, Brussel.

Van Den Berge K., Gouwy J., Berlengee F., Vansevenant D. (2013). Oriënterende verkenning naar de stadsvos in Vlaanderen. Rapporten van het Instituut voor Natuur- en Bosonderzoek 2013 (INBO.R.2013.1336286). Instituut voor Natuur- en Bosonderzoek, Brussel.

Vandevelde M., Higgins R.J., Oevermann A. (2012). Inflammatory diseases. In: M Vandevelde, R.J. Higgins,

A. Oevermann (editors). *Veterinary Neuropathology: Essentials of Theory and Practice*. John Wiley & Sons, Chichster, 60-61.
Verin R., Forzan M., Schulze C., Rocchigiani G., Balboni A., Poli A., Mazzei M. (2019). Multicentric molecular and pathological study on canine adenovirus type 1 in red foxes (*Vulpes vulpes*) in three European countries. *Journal of Wildlife Diseases 55*, 935-939.
Walker D., Fee S.A., Hartley G., Laermount J., O'Hagan M.J.H., Meredith A.L., Bronsvoort B.M.D.C., Porphyre T., Sharp C.P., Philbey A.W. (2016a). Serological and molecular epidemiology of canine adenovirus type 1 in red foxes (Vulpes vulpes) in the United Kingdom. *Scientific Reports 6*, 1-6.
Walker D., Abbondat, E., Cox A.L., Mitchell G.B.B., Pizzi R., Sharp C.P., Philbey A.W., (2016b). Infectious canine hepatitis in red foxes (Vulpes Vulpes) in wildlife rescue centres in the UK. *Veterinary Record 178*, 421.
Wilcock, B.P., Njaa B.L. (2016) Special senses. In: M.G. Maxie (editor). *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*. Sixth edition, St. Louis MO. Elsevier Ltd, 452-453.
Willis A.M. (2000). Canine viral infections. *The Veterinary Clinics of North America: Small Animal Practice 30*, 1119-1133. doi:10.1016/s0195-5616(00)05010-5.

**Uit het verleden**

## Ondankbare klant

Zoals het hoort in dierenfabels krijgen dieren menselijke trekjes toebedeeld. Ze voeren het woord, oefenen beroepen uit, enz. Zo spelen dieren zelfs dierenarts in een paar fabelgedichten van La Fontaine

We zien, onder meer, hoe een ooievaar een delicate operatie uitvoert bij een gulzige wolf met slokdarmobstructie: een been was er in blijven steken. Met zijn lange bek en hals slaagt hij er in het 'vreemd voorwerp' te verwijderen. Maar dan vraagt de operateur het ereloon. 'Wat? Hoe durf je!', repliceert de wolf: 'ik heb mijn slokdarm teruggetrokken uit jouw hals!'

In een andere fabel van dezelfde La Fontaine prijst een wolf zich aan om dieren te verlossen van hun lijden. Zijn specialiteit is … uiteraard chirurgie.

Fables de La Fontaine. Le loup et la cigogne, door Marc Mammerickx gepresenteerd in La Semaine *Vétérinaire 769, 18 maart 1995*.

Luc Devriese. Met dank aan Paul Tavernier

## REINIGEN VAN DE PENIS EN KOKER BIJ HET PAARD

### VRAAG

***"Wat is het advies met betrekking tot het reinigen van de penis en koker van een paard (Hoe, frequentie, welke producten worden daarvoor gebruikt)?"***

### ANTWOORD

De talgklieren in de koker vormen talg. Wanneer de talg zich met vuil vermengt, ontstaat smegma, wat kan leiden tot irritatie. Tekenen van irritatie zijn een gezwollen koker of smegma aan de binnenkant van de benen.

De frequentie voor het reinigen van de penis verschilt van paard tot paard en hangt ook af van de ouderdom van het paard. Bij oudere ruinen wordt aangeraden dit maandelijks te doen. Aanvankelijk verloopt het reinigen meestal moeizaam en is sedatie vaak een noodzaak. Acepromazine (ACP) is daarbij het aangewezen product. De dosering gaat van 0,05 mg/kg tot 0,1 mg/kg IV. Na toediening dient vijftien minuten gewacht te worden. Het paard is dan licht gesedeerd en schacht uit. Als de penis echter wordt vastgenomen, schacht hij vaak terug in. Wanneer dit gebeurt, is het aan te raden een bijkomende alfa2-agonisten toe te dienen, i.e. detomidine, romifidine of xylazine.

Het is raadzaam een spons (waarvan het gebruik uiteraard beperkt wordt tot één paard) en lauw water te gebruiken. Bij goedgekeurde dekhengsten of internationaal transport van paarden wordt soms een 4%-chlorhexidine- of isobetadine-oplossing gebruikt. Echter, indien dat niet nodig is, wordt de toediening ervan afgeraden, omdat de oplossing tot nog meer irritatie kan leiden

Het paard moet wennen aan deze behandeling. Wanneer het proces frequent herhaald wordt, reageren de ruinen/hengsten meestal beter. Bij massage van de koker met lauw water schachten ze vaak spontaan uit.

Dierenarts D. Van Hende
Vakgroep Heelkunde en Anesthesie van de Huisdieren, Faculteit Diergeneeskunde, Universiteit Gent,
Salisburylaan 133, B-9820 Merelbeke



### Kat dood

Sinds de kat dood is,
Moet ik minder stofzuigen
En vaker huilen.

Johan Sebastiaan Stuer.
In Humo 23 januari 2020

# *Hyalomma rufipes* (reuzenteek) nu ook aangetroffen in België

**E. Claerebout, N. De Wilde, S. Casaert**

Laboratorium voor Parasitologie, Faculteit Diergeneeskunde, Universiteit Gent, Salisburylaan 133, B-9820 Merelbeke

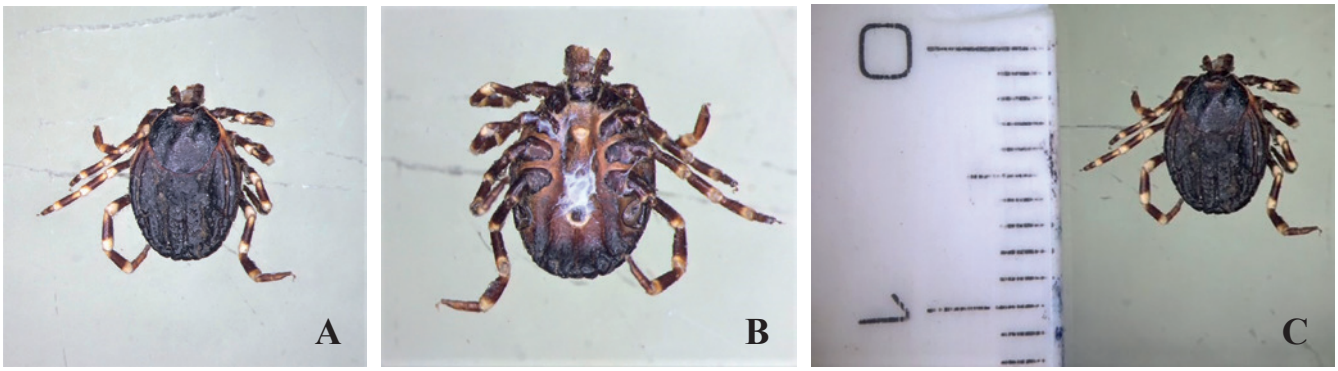
Edwin.claerebout@ugent.be

In juli 2020 werd voor het eerst een *Hyalomma* teek gevonden in België. Het betrof een volwassen vrouwelijke teek, die aangetroffen werd op een paard in het Oost-Vlaamse Zevergem (De Pinte). De 'reuzenteek' is herkenbaar aan de grootte (ongeveer 2-3 x zo groot als de veel voorkomende 'schapenteek' *Ixodes ricinus*) en aan de gestreepte poten (Figuur 1). In tegenstelling tot de teek *Dermacentor reticulatus,* die plaatselijk voorkomt in België (Cochez et al., 2012), is het rugschild van *Hyalomma* niet gevlekt. Op basis van een DNA test (PCR op het 16S rDNA gen, gevolgd door kloneren en sequeneren van het PCR product) werd de teek geïdentificeerd als *H. rufipes*, die nauw verwant is aan *H. marginatum.*

*H. marginatum* en *H. rufipes* teken zijn inheems in Noord-Afrika, Azië en Zuid- en Oost-Europa en worden occasioneel in Noord-West-Europa ingevoerd door trekvogels (larven en nymfen). Meestal kunnen deze teken hier niet overleven door de koude en vochtigheid. In warme, droge jaren kunnen *Hyalomma*-nymfen zich uitzonderlijk ontwikkelen tot volwassen teken. In de afgelopen jaren werden occasioneel volwassen *Hyalomma*-teken aangetroffen in Nederland (Nijhof et al., 2007; Uiterwijk et al., 2020) ) en in Duitsland (Chitimia-Dobler et al., 2016). Voor zover ons bekend, is dit de eerste waarneming van *Hyalomma rufipes* in België. Eerder werd wel reeds *H. aegypticum* aangetroffen, een tekensoort die geïmporteerd wordt met schildpadden (Obsomer et al., 2013)

*H. marginatum* en *H. rufipes* parasiteren vooral hoefdieren, zoals runderen, wilde herkauwers en paarden, maar zullen slechts uitzonderlijk de mens aanvallen. In tegenstelling tot de inheemse teken, die hun gastheer (dier of mens) herkennen op basis van de geur en zich vanuit een hinderlaag op hun gastheer laten vallen ('zoekende teken'), behoren *H. marginatum* en *H. rufipes* tot de 'jagende teken'. Zij identificeren hun slachtoffers op het zicht en gaan dan actief naar de gastheer toe rennen.

*H. marginatum* en *H. rufipes* teken kunnen verschillende ziekteverwekkers overdragen, zoals Krim-Congovirus*, Rickettsia spp*. en *Theileria equi.* Krim-Congovirus kan virale hemorragische koorts (Krim-Congokoorts) veroorzaken bij de mens, wat met een acuut en ernstig ziektebeeld kan gepaard gaan, gekenmerkt door bloedingen en hoge koorts, met een dodelijke afloop in 5-40% van de infecties. *Rickettsia spp*. zijn bacteriën die vlekkenkoorts ('spotted fever') of tekenkoorts veroorzaken bij de mens. Een infectie met deze bacteriën kan koorts, hoofdpijn, spierpijn en een karakteristieke huiduitslag veroorzaken. *T. equi* veroorzaakt piroplasmose bij paarden, een potentieel dodelijke ziekte die gepaard gaat met koorts, slaapzucht (lethargie) en bloedarmoede (anemie). De *H. rufipes*-teek die in Vlaanderen werd gevonden, testte negatief voor *T. equi.* Er werd wel DNA aangetroffen van *Rickettsia.* Op basis van het geamplificeerde stukje DNA kon niet uitgemaakt worden of het om *Rickettsia aeschlimannii* of *R.*




**Figuur 1. Volwassen vrouwelijke *Hyalomma rufipes*-teek. A. dorsaal aanzicht; B. ventraal aanzicht; C. afmeting.**

*massiliae* ging. Omdat geen RNA kon verzameld worden van deze teek, kon de aanwezigheid van het Krim-Congovirus niet onderzocht worden. Sinds 2012 zijn ongeveer vijftig *Hyalomma*-teken die in Noord-West-Europa zijn gevonden, getest voor het Krim-Congovirus. Alle testen waren negatief, geen van deze teken had het virus bij zich (https://www.tekenradar.nl/nieuws/actueel-op-tekenradar-nl?action=ShowItem&ciId=1550&state=online).

## REFERENTIES


Chitimia-Dobler L., Nava S., Bestehorn M., Dobler G., Wölfel S., (2016). First detection of *Hyalomma rufipes* in Germany. *Ticks and Tick-borne Diseases 7*, 1135-1138

Cochez C., Lempereur L., Madder M., Claerebout E., Simons L., De Wilde N., Linden A., Saegerman C., Heyman P., Losson B., (2012). Foci report of indigenous *Dermacentor reticulatus* populations in Belgium and a preliminary study on associated babesiosis pathogens. *Medical and Veterinary Entomology 26*, 355-358

Nijhof A.M., Bodaan C., Postigo M., Nieuwenhuijs H., Opsteegh M., Franssen L., Jebbink F., Jongejan F., (2007). Ticks and associated pathogens collected from domestic animals in the Netherlands. *Vector-borne and zoonotic diseases 7*, 585-595

Obsomer V., Wirtgen M., Linden A., Claerebout E., Heyman P., Heylen D., Madder M., Maris J., Lebrun M., Tack W., Lempereur L., Hance T., Van Impe G., (2013). Spatial disaggregation of tick occurrence and ecology at local scale as preliminary step for tick-borne diseases spatial surveillance: general framework and health implications in Belgium. *Parasites & Vectors 6*, 190

Uiterwijk M., Ibáñez-Justicia A., Sprong H., Overgaauw P., Nijsse R., Dabekaussen C., Stroo A. (2020). *Hyalomma* teken in Nederland. *Tijdschrift voor Diergeneeskunde*, KNMvD.

# EXAMENUITSLAGEN EERSTE EN TWEEDE ZITTIJD 2019-2020

**Behaalden de academische graad van dierenarts**

## Optie herkauwers

Aneca Milo, Baert Rein, Bannink Jet, Billiet Roxanne, Botermans Robert, Capiau Matthias, Clinquart Justine, Collet Gilles, Coppens Tom, Coudenys Fien, Croockewit Dominique, Davy William, De Baets Jakob, Debbaut Roos, Decorte Bregt, Deschoemaeker Amber, De Valck Astrid, Droesbeke Brecht, Engelhardt Hannah, Forier Liesbeth, Geeroms Nele, Hansen Line, Helmers Vera, Jonckheere Marie, Jourquin Stan, Lannoo Brecht, Lannoo Felix, Lardenoit Glenn, Lazzarotto Priori Nathascha, Leenaerts Hannelore, Limonard Iris, Lowie Thomas, Meuleman Sophie, Pelgrims Alyzee, Pieters Jolien, Poppe Valerie, Samyn Joyce, Schaele Sofie, Schmittgens Judith, Stein Lisa, Triepels Sanne, Van Acoleyen Gwenny, Van Den Abeele Dominiek, Van Eetvelt Ann, Van Hoof Loppe, van Maanen Charlotte, Van Parys Kristof, Van Puyvelde Justine, Verlinden Bram, von den Driesch Christian, Vos Suzanne Maria, Wuyts Charlotte, Wuyts Jochem, Wylin Rutger

## Optie varken, pluimvee en konijn

Cobbaert Jessy, Donkers Dries, Matthys Thomas, Misplon Janneke, Rommens Yana, Van Acker Carine, Vandenabeele Jordy, Verhulst Robin, Warlop Juul

## Optie gezelschapsdieren

Adams Eline, Alon Anoek, Ampe Laure-Ann, Attasio Marije Pauline Johanna, Audiens Evelyne, Barin Tiffany, Bernaerts Soline, Biets Heleen, Boelhouwer Mara, Brakelé Manuela, Brand Alexander, Breynaert Arno, Castelain Donatienne, Ceyssens Elisa, Christiaens Bieke, Clevers Stéphanie, Cleymans Joy, Cools Tom, Couder Kiani, Dauwen Marlies, De Backer Anouchka, de Bie Pascaline, de Froe Heleen, De Ketelaere Matthias, De Landtsheer Perrine, De Raeymaeker Nicolas, De Vree Jeremy, De Vriendt Yana, Debie Chiara, Decock Pieter, Decubber Selinde, Dekker Anne-Linde, Derdeyn Robin, Dielissen Cornelia, Donga Hedwig Iris, Feys Laura, Filina Maryna, Gagea Alexandra, Geerts Gitte, Glazemaekers Evelien, Goossens Juliette, Graafmans Niels, Gras Pien, Hartings Esmee, Henkel Elisabeth, Heremans Inge, Herreman Lauren, Holsteens Jessica, Huisman Elza, Huurman Floortje, Iqbal Halima, Jubbega Marloes, Kempen Melanie, Kestelijn Jasmine, Kints Inka, Krekels Josefien, Kroese Silvie, Lammens Naomi, Lefever Jason, L'Oeuille Fauve, Loyson Emma, Maes Nathalie, Major Amber, Martelli Sebastian, Matthysen Tine, Meijer Charlotte, Mevissen Britt, Mulder Job Jesse, Musters Camiel, Nurmohamed Gwendolyn, Ooms Sam, Peeters Valérie, Pennings Michelle, Peters Natalie, Raeijmaekers Pauline, Raets Lisa, Reindsen Britt, Roosen Iris, Rosiers Lissa, Sannen Jeroen, Schoofs Sarie, Schuddinck Joni, Sneyers Lisa, Steenhorst Loïs, Talloen Louise, Uten Andreas, van Breukelen Mignon Maria, Van de Voorde Amber, Van den Block Jonas, van den Hurk Lotte, van der Lee Mathilde Anna, Van Doren Larissa, van Duijn Liesanne, Van Gampelaere Birgit, Van Hautegem Lieselot, van Heerdt Sharon, Van Laer Kato, Van Laethem Charlene, Van Looy Marie, Van Mulders Laurens, Van Sieleghem Marie, Vanden Borre Florence, Vanden Broecke Ellen, Vandenbussche Tom, Vandermeeren Aina, Vanrobaeys Ine, Veldhuijsen Kim, Veragten Femmie, Verdoodt Fien, Verhelst Ellen, Vermeiren Yoni, Vermeulen Valérie, Verryssen Sofie, Verschueren Aurélie, Vervaeke Pieter, Vincken Maarten, Vonk Marloes, Voorboom Cornelis Koenraad, Watty Lara, Welters Marie, Winnepenninckx Emilie, Zwaenepoel Julie

## Optie paard

Baauw Anna, Bartels Tara, Blockx Zoé, Bollen Celine, Bouckaert Stefanie, Brants Olivia, Bregman Seline, Buelens Marieke, Buschmann Eva, Buyck Camille, Calewaert Amber, Callens Ruben, de Bakker Anna, Debeuf Tobias, Deboeck Julie, Defever Lars, Depoortere Hélène, Deprez Manon, De Vos Annelies, D'hooghe Julie, Endres Kim, Entjes Syèn, Esprit Lucas, Evers Elien, Geel Astrid Corine, Gerth Kirsten, Greiner Isabelle, Groeneveld Dianne, Havik Wies, Herygers Melissa, Héylen Femke, Huitema Sita, Kamps Melissa, Laureys Britt, Liwing

Fanny Wenche, Maes Elza, Manneveld Valery, Meeremans Marguerite, Meijer Florin, Meijne Amy, Mievis Astrid, Naudts Laura, Noelanders Jasmine, Ostermann Carmen, Peeters Julie, Piccavet Florence, Pless Tineke, Polfliet Ellen, Roose Jolien, Rozemeijer Renée, Selfslagh Gaëlle, Simons Hanne, Stael Janne, Straetmans Kyara, Tavernier Justine, van Brug Eleonora Maria, Van den Berg Quinty, Van Gelder Kimberly, van Heule Machteld, Van Mol Bram, Van Roosenbroek Sophie, Verhoeven Yordi, Verstappen Stefanie, Verstraete Margo, Vos Jannick René

**Optie onderzoek**

Cokelaere Dries, Coppens Lien, Courtens Laura, De Bruyne Liana, Franken Esther, Kariza Ona, Luyckx Severine, Van den Abeele Sharon, Van Hoorde Aline

**Uit het verleden**

## ‘Zotte’ schapen in Vossem bij Leuven (1445 – 1460)

Blijkens de bewaard gebleven rekeningen van de landbouwdomein Vossem van het Brusselse Apostelengodshuis waren sommige jaren ‘slecht’ omdat er hoge sterfte optrad in de schapenkudde die jaarlijks zorgde voor vijf tot twintig percent van de inkomsten. Rond het midden van de vijftiende eeuw staan er, met tussenpozen, vier jaren genoteerd met tegenvallende resultaten. Een aandoening aangeduid als ‘zotte schapen’ verspreidde zich snel in de kudde. Lammeren moesten aangekocht worden om de verliezen te compenseren. In normale jaren lag de sterfte rond 5% en overtrof het aantal nieuwgeboren lammeren veruit de sterfte.

Als oorzaken komen in aanmerking: coenurus aantasting in de hersenen (door larvaire cysten van *Taenia* soorten), scrapie (toen al?) en listeriose. Geen enkele aanduiding wordt gegeven die meer specifiek naar een bepaalde ziekte kan wijzen. Dat was nog minder het geval in het rampjaar 1465-1466 toen de hele kudde verkocht werd omdat er schapen waren die ‘ongeve (ongaaf) ende besmet waeren’ en men vreesde dat ze ‘al besmet’ zouden worden. In andere jaren waren belangrijke verliezen te wijten aan roofzuchtige wolven of legerbenden.

Uit: Blockmans, W. (1970). De rendabiliteit van de schapenteelt in Brabant tijdens de 15de eeuw. *Bijdragen tot de Geschiedenis 53*, 113-125.

Luc Devriese. Met dank aan Marc Hanson

# IDEXX **Catalyst One®**

**Voortdurende medische innovaties** verzekeren u van alle klinisch-chemische testen die u nodig heeft


Meer weten? Ga naar **www.idexx.be/COVDT** en vraag vrijblijvend een offerte aan.



IDEXX

# EICKEMEYER® 15-jarig jubileum

## *Medische beeldvorming inspiratie*

**Het einde van het jaar is weer in zicht. Ook deze laatste maanden van het jaar staan we voor u klaar! Mocht u vragen hebben of een demonstratie wensen, neemt u dan contact met ons op.**


### Laser Platinum 4

- Pijnloze behandeling voor beter en sneller herstel
- Te gebruiken bij diverse aandoeningen
- 4 golflengtes (660, 800, 905 en 980 nm)
- 24 Watt continuous wave en 28 Watt super pulse

### HIRAY dental wandmodel mét

- Compact en gebruiksvriendelijk
- Scherpe, heldere beelden
- Inclusief starterkit met image plates
- Inclusief veterinaire software
- Optioneel leverbaar met dentale rontgenbuis

### DigiVet® CR Dentale Reader


### Examion portable large


- Het panel weegst slechts 3,7 kg
- Met auto exposure detectie systeem
- WiFi tussen panel en laptop
- 35 cm x 42 cm actief oppervlak
- Inclusief software en laptop
- Geleverd in koffer

**TELEFOON 0031 345 589400**

www.eickemeyer.nl

veterinary technology for life

Eickemeyer®