ASPECTOS VETERINARIOS DEL PROGRAMA DE REINTRODUCCIÓN DE LA NUTRIA EUROASIÁTICA (*LUTRA LUTRA*): HEMATOLOGIA, ANESTESIA Y CONTROL DE LA RESPUESTA DE ESTRÉS

Memoria presentada por Jesús Fernández Morán
para optar al grado de Doctor en Veterinaria

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Departament de Biologia Cel·lular, de Fisiologia i d'Immunologia

Unitat de Fisiologia Animal (Veterinària)

XAVIER MANTECA I VILANOVA, Professor Titular de Fisiologia del Departament de Biologia Cel-lular, Fisiologia i Immunologia de la Facultat de Veterinària de la Universitat Autònoma de Barcelona,

CERTIFICA:

Que la memoria titulada **"Aspectos veterinarios del programa de reintroducción de la nutria euroasiática (*Lutra lutra*): hematología, anestesia y control de la respuesta de estrés"** presentada per Jesús Fernández Morán per optar al grau de Doctor, ha estat realitzada sota la seva direcció i, considerant-la finalitzada, autoritzen la seva presentació per tal que sigui jutjada pel tribunal corresponent. La lectura de l´esmentat treball es durà a terme al Departament de Medicina i Cirugia Animals de la Universitat Autònoma de Barcelona, tenint com a tutor el Dr. Felix Garcia Arnas.

I perquè consti als efectes oportuns, signo el present certificat a Bellaterra, a 21 d' Juny de 2003.

X. Manteca

Edifici V-Campus de la UAB-08193 Bellaterra (Cerdanyola del Vallès)-Barcelona, Spain
Tel.: 34- 9358121898- Fax: 935812006
u.vet.fisiología@cc.uab.es
www.uab.es

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El programa de reintroducción de la nutria en Cataluña surgió como una iniciativa única y vanguardista de la Asociación de Amigos del Parque de Aiguamolls de l´Empordà (APNAE), formada por cerca de mil socios, siendo los padres y promotores de la idea los naturalistas Jordi Sargatall y Deli Saavedra. A estos dos conservacionistas empordaneses y a su empeño, les debemos el poder volver a disfrutar de las nutrias en los ríos Muga y Fluvià, con todas sus implicaciones positivas para el ecosistema.

A pesar de los escollos encontrados en el camino y una vez completados los estudios de viabilidad de las zonas protegidas, se procedía a la elaboración de los planes de trabajo con vistas a la captura y suelta de los ejemplares que habían de ser los nuevos "colonos" de la zona. Tras numerosas reuniones con el entonces gerente del Zoo de Barcelona Enric Mas y el Director técnico Jaume Xampeny se acordó que el Servicio Veterinario del Zoo de Barcelona se encargaría de aportar la infraestructura veterinaria necesaria durante todas las fases del proyecto. Gracias a ellos nuestro Zoo pudo ser participe de este proyecto de conservación tan importante y complejo que se extendería hasta el año 2000.

Desde el primer momento, fueron numerosos los trabajadores del Zoo que se volcaron en este atractivo trabajo de "puertas afuera" – o dicho de manera más sería: "de conservación *in situ*" – . Me gustaría destacar a los compañeros Conrad Enseñat, Salvador Filella, Marina Casanelles, Marga Rosell y Justo Garasa quienes constituyeron un importante apoyo en los primeros momentos difíciles de trabajo con este singular y "escapadizo" mustélido. Jordi Fábregas fotografió en algún momento cada una de las nutrias que pasaron por nuestras instalaciones. Este material de archivo constituye una valiosa fuente de información. Tampoco quiero olvidar a otros compañeros cuya ayuda fue muy importante para preparar los que serían los nuevos habitáculos de los animales recién capturados: el personal dirigido por Joan Bassas precisó de muchas horas para poder acabar a tiempo las 6 jaulas que habían de estar ocupadas por nutrias salvajes durante los siguientes años.

Transcurrió el primer año años, y los responsables del proyecto continuaron confiando en el Zoo de Barcelona, a pesar de los cambios que se producirían. El nuevo Director General del Zoo Esteve Tomás, lejos de frenar el proyecto, le aplicó un nuevo impulso, inaugurándose en el Zoo lo que sería la presentación al público de la nutria, los ecosistemas fluviales catalanes y el proyecto que se estaba llevando a cabo en el Empordà. Con fondos de la Fundació Terrotori i Paitsaje y del propio Zoo, la Presidenta del Consejo de Administració Maravillas Rojo y Esteve Tomás inauguraban la nueva instalación, una de las mejores del mundo. Puertas adentro, esta instalación era un reflejo del trabajo que se estaba desarrollando en el campo en aras de la conservación. Además, desde el año 1996, la clínica veterinaria del Zoo recibió nuevos equipamientos que hicieron posible llevar a cabo todas las tareas veterinarias necesarias durante el proyecto.

El Director técnico, Ferran Costa nos facilitó enormemente el trabajo permitiéndonos disponer siempre de todo aquello que nosotros o las nutrias necesitamos. No solamente nunca nos faltó nada en el ámbito técnico, sino que sentimos en todo momento el respaldo necesario que hace que uno se dedique en cuerpo y alma más allá de la estricta obligación profesional.

También han sido numerosos los cuidadores que me han ayudado durante este tiempo. De manera especial, Pilar Padilla y Luís Parejo, cuidadores de las nutrias en el Zoo se mostraron siempre entusiastas y solícitos ante mis reiteradas consultas y solicitudes. Sin ellos yo no habría obtenido algunas de mis preciadas muestras y miles de visitantes no habrían podido disfrutar de la inolvidable visión de nuestras nutrias nadando y jugando en su instalación.

Durante el desarrollo del proyecto, han sido cientos los voluntarios que han colaborado. No puedo citar a todos ellos pero quiero expresarles mi agradecimiento ya que sin ellos todo hubiera sido imposible. Sin embargo, durante los últimos años, contamos con un grupo de monitores y estudiantes que se encargaron del cuidado diario de las nutrias así como de la metódica toma de muestras. Su trabajo no tiene precio y creo que sus desinteresados sacrificios únicamente podrán ser entendidos como fruto de la pasión y entusiasmo por la naturaleza. Ellos son Ester, Gemma, Belén, Josep, Mari Cruz, Critina, Sandra, entre otros y a ellos les tocó

enfrentarse a momentos de pánico como cuando Ester me llamó un domingo a primera hora alarmada al comprobar que Pinto había desaparecido de su jaula.

También estoy agradecido de manera especial a todas aquellas personas que me ayudaron durante la publicación de los trabajos que se recogen a continuación: Lourdes Molina, Marta Sanmartin, Emi Pérez, Jose Luis Ruiz De La Torre, Jordi Ruiz, Deli Saavedra y Xavier Manteca, Lucy Spelman, Rafael Cebrian, Helena Marqués, Jon Arnemo, Marie Pierre Ryser-Degiorgis, Joase Domingo, Willem Schaftenaar, Eric Miller, Cheryl Asa, Carme Maté y Bengt Röcken.

Los amigos Miquel Sanllehy y Juan Cecilia a menudo me preguntaban sobre los "tachones" de los *referees* en los trabajos enviados para publicar. A base de quejas, también les hice un poco partícipes de este largo proceso. Miquel también me ayudó a la impresión final de la tesis. Por desgracia nunca lo podremos celebrar con Juan. Nos quedó una deuda pendiente que algún día acabaremos de pagar...

Desde el primer momento tuvimos la suerte de contar con el apoyo incondicional de uno de los principales expertos mundiales en la nutria. A Jordi Ruiz le avalan sus más que numerosas publicaciones sobre la materia así como sus miles de horas de estudio de campo de este mustélido. No solo nos ayudó revisando los manuscritos, sino que sus visitas al Zoo durante las primeras intervenciones constituían útiles clases de biología para un pobre veterinario de Zoo, aunque para compensarle, y en una ocasión, le tocó presenciar una escena de quirófano algo sangrienta pero exitosa.

Aunque no compartíamos faenas ya que nuestras intervenciones se solapaban, los equipos de captura y transporte de nutrias eran los responsables de que las nutrias llegaran sanas y a salvo a sus lugares de tránsito en la clínica del Zoo tan rápido como fuera posible. ¿Cuántas horas de conducción se llevaron a cabo para evitar a las nutrias viajes "estresantes" en avión? Sólo ellos lo saben. Gracias a Toni Batet, Raimon Mariné, Pons Feliu y Sergi Romero entre otros.

Gracias a todos aquellos amigos que en algún momento me han ayudado; son tantos que no podré citarlos a todos. Algunos ya Doctores se interesaban por el estado de mi tesis cada vez

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Inicié los trabajos de esta tesis doctoral porque en primer lugar, creía que disponía de una buena oportunidad de hacer ciencia y conservación y así poder aportar algún conocimiento a futuros planes similares. El segundo aspecto que me animó a lanzarme al ruedo fue conocer a mi Director, Xavier Manteca. Ahora que estoy en la recta final no hago más que aconsejar a quienes me preguntan por como hacer una tesis, sobre lo importante que es la elección de un buen Director de Tesis. Siempre que sus viajes profesionales se lo permitían estuvo ahí, apoyándome. Su papel en este trabajo final fue fundamental guiándome y animándome en los momentos en que era preciso y dejándome hacer a mi ritmo en el día a día. Sin su apoyo supongo que esto se habría quedado por el camino. Nuestros lugares de encuentro para discutir la tesis no fueron muy clásicos. Solíamos charlar al respecto mientras corríamos nuestros 10 kilómetros por la noche en la emblemática carretera de las aguas de Barcelona (lugar por otra parte frecuentado por la élite del atletismo barcelonés de fondo), por lo que si se aprecian pequeños errores ruego no se tengan en cuenta. Por lo demás quiero hacerle llegar mi pesar por los sudores, las series y fatigas que hice padecer a mi maestro, aunque seguro que le fueron recompensados en parte con las cervezas con las que brindamos al final de nuestro/s maraton/es. Nuestro lema de entrenamientos me lo

hice propio para poder finalizar esta tesis: “no pain no gain” aunque sé que no siempre se ajusta a la realidad.

Mis padres y hermanos me animaron siempre que les nombraba la tesis y la pregunta ¿ya la lees? Actuaba en mi como un resorte automático que me hacía entregarme de lleno a la tesis durante unas semanas. La ilusión que le hacía a mis padres se convirtió en una de mis principales motivaciones.

Si hay alguien quien merece mi profundo agradecimiento por poder estar hoy presentando este documento es sin duda, Marga, mi mujer. Ya sé que se ha convertido en tópico –seguro que con razón – pero sin su apoyo y labor de soporte yo no habría podido haber dedicado todas estas horas a “mí mismo”. Me ha soportado estoicamente todas estas horas inmerso en el ordenador y en mis “trabajitos” sabedora de la importancia que tenían para mí, a la vez que también ha sabido frenarme en los momentos de máxima ofuscación, recordándome que había otras cosas.

A las nutrias, mis amigas de fatiga. Las que quedaron en el camino, las que llegaron a su nuevo destino y a las que ahora corren y nadan por los ríos catalanes y franceses. Espero que todo este sacrificio se vea recompensado y que las experiencias aquí recogidas sirvan para aliviar el sufrimiento de otros animales salvajes en programas similares.

Gracias a Laika, por los momentos pasados juntos en el campo; Si te encuentras a “Cuanti”, “Petit”, “Aurora”…¡qué tengáis buenas inmersiones en los ríos!

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INTRODUCCIÓN GENERAL

1. Mustélidos-nutrias

La familia Mustelidae incluye unas 63 especies de mamíferos terrestres y/o acuáticos que habitan todos los continentes excepto Australia, Nueva Guinea, Madagascar y la Antártida. Esta familia incluye 5 subfamilias:

Mustelinae	33 especies	Comadrejas, hurones, visones
Mellivorinae	1 especie	Ratel o tejón de la miel
Melinae	8 especies	Tejones
Mephitinae	9 especies	Mofetas
Lutrinae	13 especies	Nutrias

En general se trata de mamíferos carnívoros o piscívoros (también omnívoros en algunos casos), predominantemente solitarios y terrestres. Algunas especies (nutrias y visones) son semi-acuaticos o totalmente acuáticos y han desarrollado mecanismos únicos de adaptación: visión subacuática, capa de pelo con alta capacidad de aislamiento térmico e impermeabilidad, y adaptaciones anatómicas para el desplazamiento en el agua. Al ser capaces de desplazamiento cuadrúpedo perfecto fuera del agua, las nutrias y visones son considerados por algunos autores como mamíferos morfológicamente intermedios entre los acuáticos (cetáceos y focas) y los terrestres (Estes 1996).

En la Península ibérica habitan los siguientes mustélidos (Blanco & González 1992):

NOMBRE CIENTÍFICO	CASTELLANO	CATALÁN	GALLEGO	EUSKERA
Mustela nivalis	Comadreja	Mostela	Denociña	Erbinudea
Mustela erminia	Armiño	Ermini	Erminio	Erbinude zuria
Mustela putorius	Turón	Turó	Furón bravo	Ipurtatsa
Mustela lutreola	Visón europeo	Visó europeu	Visón	Ur-ipurtatsa
Mustela vison	Visón americano	Visó americà	Visón	Bisoi amerikarra
Martes martes	Marta	Marta	Marta	Lepahoria
Martes foina	Garduña	Fagina	Garduña papalba	Lepazuria
Lutra lutra	Nutria	Llúdriga	Lontra	Igaraba arrunta
Meles meles	Tejón	Teixó	Porco Teixo	Azkonarra

Dentro de la Subfamilia *Lutrinae* de los mustélidos, existen cinco géneros: *Lutra, Lontra, Aonyx, Enhydra, y Pteronura,* aunque algunos autores reconocen otros como *Amblonyx, Hydrictis y Lutreogale*. El género *Lutra,* al que pertenece la nutria euroasiática o europea (*Lutra lutra*), se distribuye por Europa, Afrecha y Asia. Sus dimensiones son variables. En la Península Ibérica las hembras adultas suelen pesar entre 4,5 y 7,0 kg y medir entre 95 y 110 cm (longitud total, con la cola incluida) mientras que los machos pesan entre 6,5 y 10 kg y miden entre 105 y 120 cm. La anatomía esta totalmente adaptada al medio acuático: cuerpo alargado e hidrodinámico, pies palmados, cola larga y aplanada, piel aislante, orificios nasales, ojos y oídos en posición superior del cráneo y presencia de vibrisas táctiles alrededor del hocico y en los brazos (Baitchman & Kollias 2000; Krukk 1996; Ruiz-Olmo 2001; Ruiz-Olmo & Delibes 1999).

Aunque la nutria estaba presente en toda Europa, recientemente sus poblaciones han disminuido en determinadas áreas (las más pobladas y contaminadas), hasta quedar restringida a

las áreas más solitarias y salvajes, y por lo tanto mejor conservadas. Entre las principales causas que motivaron este declive poblacional en la especie se citan: la contaminación, la destrucción de sus hábitats, la disminución y la alteración de los recursos alimentarios, la mortalidad de origen humano (furtivismo, atropellos, perros), la fragmentación de las poblaciones y la sobre-explotación del agua como recurso natural (Ruiz-Olmo 2001).

Durante los años 1984 y 1985, se realizó un estudio en profundidad sobre la distribución de la nutria en España (Delibes 1990) que demostró el declive de la especie en la vertiente mediterránea, en las áreas más industrializadas, así como en las más agrícolas. La nutria había desaparecido de la mayor parte de Cataluña, encontrándose recluida a zonas periféricas del norte, al oeste y al sur. Además, entre 1986 y 1989 desaparecieron las poblaciones de los ríos Algars, Montsant, Muga y Alto Segre. A partir de los años 1988/89, la nutria inició un proceso gradual de recuperación natural en Cataluña (Ruiz-Olmo & Gonsalbez 1988; Ruiz-Olmo 2001).

2. Reintroducción de la nutria

La reintroducción de especies animales y plantas constituye una herramienta muy útil y cada vez más empleada en el campo de la conservación. Según la Unión Internacional para la Conservación de la Naturaleza (IUCN 1995), se define como reintroducción a cualquier intento de restablecer una especie en un área que fue en algún momento parte de su distribución histórica, pero de la cual ha sido extirpada o se extinguió. Según la citada referencia, los objetivos de este tipo de programa consisten en aumentar las probabilidades de supervivencia de una especie a largo plazo; restablecer una especie clave en un ecosistema; mantener y/o restaurar la biodiversidad natural; proveer beneficios económicos a largo plazo a la economía local y/o nacional; promover la toma de conciencia de la conservación; o alguna combinación de ellos. En este contexto y durante las últimas décadas se han desarrollado numerosos programas de reintroducción de mamíferos (algunos exitosos, otros fallidos) entre los que podemos destacar los siguientes (Bush et al 1993; Clark et al. 1994; Kleiman 1996; Reading & Miller 1994; Sjöasen 1997):

Especie	**Nombre en inglés**	**Nombre científico**	**Lugar**
Hurón de patas negras	Black footed ferret	*Mustela nigripes*	EEUU
Nutria americana	North American river otter	*Lontra canadensis*	EEUU
Orix de Arabia	Arabian Oryx	*Oryx leucorix*	Arabia
Titi León	Golden lion tamarin	*Leontopithecus rosalia*	Brasil
Lobo rojo	Red wolf	*Canis rufus*	EEUU
Bisonte americano	American bison	*Bison bison*	EEUU
Oso pardo	Brown bear	*Ursus arctos*	Francia-España
Bisonte Europeo	European wisent	*Bison bonasus*	Polonia
Nutria europea	Eurasian otter	*Lutra lutra*	Inglaterra, España
Hutia	Jamaican hutia	*Geocapromys brownii*	Jamaica

En el caso de la nutria, se han llevado a cabo numerosos y exitosos proyectos de reintroducción. En los Estados Unidos, más de 4.000 ejemplares han sido trasladados durante los últimos años, de los cuales la mayoría procedían de los estados de Missouri y Louisiana (Serfass, comunicación personal). En Carolina del Norte, desde 1990 hasta 1995, se introdujeron 267 ejemplares (Summer, comunicación personal). En Pennsylvania, inicialmente se reintrodujeron 75 ejemplares de nutria capturadas en los estados de Louisina, New York, Michigan y New Hampshire; posteriormente se amplió el número de ejemplares y en la actualidad se continúan reforzando algunas poblaciones. Hasta la fecha se han reforzado las poblaciones de nutria mediante programas de reintroducción en 17 estados de EEUU y una provincia de Canadá (Kimber & Kollias 2000). En Europa, se han llevado a cabo programas de menor magnitud pero igualmente exitosos en el Reino Unido, Suecia y España (Jessop & Cheyne 1992; Saavedra & Sargatal 1998; Serfass et al. 1996; Sjöasen 1997) y actualmente acaba de iniciarse un proyecto similar en Holanda con nutrias provenientes de otros países europeos (Jansman, comunicación personal).

Entre 1995 y el 2000, se llevó a cabo en Cataluña el Plan de Reintroducción de la nutria (*Lutra lutra*) (PRNC), mediante el cual se liberaron en las cuencas de los ríos Muga y Fluviá hasta un total de 42 individuos procedentes de Extremadura, Asturias y Portugal, gracias a convenios establecidos por la Generalitat de Cataluña (Ruiz-Olmo 2001; Saavedra & Sargatal 1998). Desde el primer momento se establecieron las bases para evitar la propagación de enfermedades infecciosas tanto en los animales reintroducidos como en la fauna local del entorno como recomienda Griffith et al. (1993). También se acordó establecer protocolos de trabajo que redujeran al mínimo el estrés y el sufrimiento de los animales capturados y trasladados.

3. Valores de referencia (hematologia-bioquímica)

La obtención de valores fisiológicos de referencia para una especie resulta importante ya que permite una mejor valoración del estado sanitario de los animales (Kimber & Kollias 2000; Meyer et al. 1992; Partridge 1995). Además, ciertos parámetros pueden sufrir variaciones en situaciones de estrés o de un manejo incorrecto (Mc Williams & Thomas 1992; Serfass et al. 1993; Whittington & Grant 1995). Asi pués, los valores de referencia permiten llevar a cabo una buena valoración general del estado de los animales o de las técnicas empleadas para su manipulación (captura, traslados, adaptación, intervenciones quirúrgicas y liberación) durante un proyecto de translocación, como el llevado a cabo con la nutria en Cataluña (PRNC).
Hasta la realización de este trabajo, existía poca información sobre aspectos fisiológicos de la nutria (Vogt 1994). En lo referente a los valores o intervalos de referencia para los parámetros hematológicos y bioquímicos, únicamente contábamos con una investigación llevada a cabo en un centro de rescate de Gran Bretaña (Lewis et al. 1998). Sin embargo, en este estudio se emplearon diferentes regímenes anestésicos así como técnicas de laboratorio no homogéneas que pudieron haber resultado en valores divergentes. Por otra parte podrían existir diferencias hematológicas en la población de nutrias estudiadas (escocesas) con respecto a las nutrias ibéricas. En cuanto a otras especies de nutrias, los estudios existentes eran muy limitados, por lo que los resultados obtenidos en este trabajo podrán ser comparados en el futuro con otras

poblaciones para las que todavía no existan valores de referencia (Baitchman & Kollias 2000; Tocidlowski et al. 2000; Williams & Pulley 1983).

4. Anestesia

Aunque las nutrias no son animales de gran tamaño y no son consideradas animales muy peligrosos, su manipulación sin protección adecuada es arriesgada para ellas y para el personal involucrado en su manejo. Las nutrias pueden ser manejadas con lazos, redes, pinzas de cuello, o jaulas de contención o curas (*squeeze cages*) pero siempre deben tomarse precauciones, pues poseen una dentadura muy fuerte y afilada. Además son extremadamente ágiles y rápidas a la hora de escapar o morder. Por este motivo, independientemente de la técnica empleada se deben proteger las manos con guantes de cuero de gran grosor.

Uno de los aspectos fundamentales en el PRNC era el disponer de una técnica anestésica adecuada, segura, fiable y asequible para el trabajo tanto de campo como en el zoológico. Existían pocas referencias científicas sobre el empleo de anestésicos en nutrias salvajes. Durante la ejecución del proyecto de reintroducción se anestesiaron más de 40 nutrias en más de 120 ocasiones ya que cada vez que un animal debía ser examinado o manipulado era convenientemente sedado con el fin de no causarle un estrés innecesario. Por otra parte, se decidió la colocación intraperitoneal de un emisor de radiolocalización en cada animal lo que permitiría su posterior seguimiento tras la liberación. Dicha intervención, requería una técnica anestésica rápida, segura, eficaz y reversible que tuvo que ser estudiada y desarrollada durante el proyecto.

La anestesia en la nutria americana ha sido especialmente estudiado por Spelman (Spelman 1999). Normalmente se suelen emplear combinaciones de benzodiazepinas (diazepam, midazolan, zolazepam), alfa-2-agonistas (xilacina, medetomidina), y disociativos (ketamina, tiletamina). Algunas de estas combinaciones pueden ser antagonizadas parcial o totalmente mediante tolazolina, idazoxan, yohimbina, atipamezol, o flumazemil (Spelman 1999). Aunque el uso de estas combinaciones estaba ampliamente documentado en la nutria americana, existían pocas referencias en la nutria eurasiática (Arnemo 1990; Jalanka & Rocken 1990; Lewis et al. 1998).

Además, la anestesia de la nutria puede causar complicaciones como: depresión respiratoria (apnea, bradipnea, taquipnea, hipoxia), hipertermia,hipotermia, bradicardia, taquicardia, falta de miorelajación y recuperación traumática (Reuther & Brandes 1984; Spelman 1999).

5. Estrés

El término “estrés” fue introducido en 1949 por Hans Seyle para referirse a una carga o presión psico-somática con repercusiones patológicas. Dicho de otro modo, el estrés sería una respuesta inespecífica a todos los estímulos que trastornan la homeostasis. Esta respuesta tendría tres fases diferenciadas: alarma, resistencia y extenuación o agotamiento (Seyle 1973).

Posteriormente Mason realizo varias contribuciones importantes en relación al concepto de estrés. Demostró que la respuesta de estrés depende de los componentes psicológicos del estímulo estresante y que la respuesta al estrés podía variar en función del estimulo estresante, además de resaltar la importancia de los aspectos comportamentales en el estrés (Mason 1968 a,b; 1971).

Un determinado estimulo ambiental es estresante en la medida que es percibido como una amenaza para la homeostasis del individuo, por lo que la respuesta al estrés depende tanto de las características del estimulo como de las características del individuo en cuestión. Los estímulos pueden clasificarse según sus características cualitativas (térmico, químico, visual, etc.) o por su intensidad y temporalidad (frecuencia, duración y regularidad o secuencia) (Broom 1993).

El que un estimulo sea percibido como estresante depende, en parte, de la denominada componente psicológica o emocional (Cabanac 1987), la cual depende a su vez de la experiencia previa del individuo. Por lo tanto, un estimulo es estresante en la medida en que resulta impredecible e incontrolable y esto, a su vez, depende de la experiencia previa del animal con dicho estimulo o con estímulos similares (Wiepkema 1987).

La respuesta al estrés se inicia con la liberación de CRF (Corticotropin Releasing Factor) (Dunn & Berridge 1990) a partir del núcleo paraventricular del hipotálamo (PVN) y del núcleo central del de la amígdala (CeA) (Chapell et al 1986). El CRF actúa sobre el eje simpático-adrenal (SA) y sobre el eje hipofiso-adrenal.

La rama simpática del sistema nervioso autónomo, cuyo neurotransmisor principal es la norepinefrina, inerva la medula adrenal que a su vez, libera norepinefrina, epinefrina y dopamina al torrente sanguíneo. La epinefrina y norepinefrina pueden actuar sobre tres tipos de receptores (α, β_1 y β_2) produciendo diversos efectos tales como: vasoconstriccion, taquicardia, incremento de la fuerza de contraccion del miocardio, relajacion intestinal, contraccion del esfinter de la vejiga, glucogenolisis, lipolisis, etc. entre otros (Guyton 1992). La activacion del eje SA esta controlada directamente por el CRF (Brown et al 1982).

Otro efecto importante del CRF es la estimulacion de la liberacion de hormona adrenocorticotropa (ACTH) por parte de la adenohipofisis (Oliverio 1987), la que a su vez estimula la secrecion de glucocorticoides por parte de la corteza adrenal (Guyton 1992). Estos tienen un gran numero de acciones, y practicamente todas las celulas nucleadas del organismo tienen receptores para ellos (Munck et al 1984).

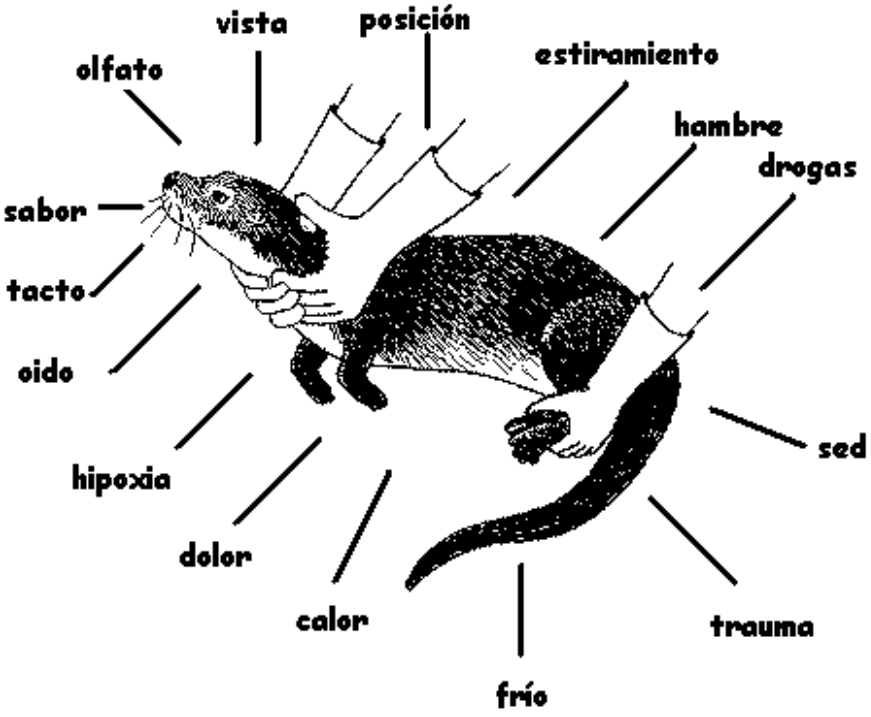
La activación del hipotálamo causa la estimulación simpática de la médula adrenal, la cual responde incrementando la liberación de catecolaminas (epinefrina y norepinefrina). El resultado final es un incremento en las frecuencias cardiaca y respiratoria , y en la presión arterial, además de otros efectos. La constricción de vasos viscerales y periféricos deriva un mayor flujo sanguíneo a los vasos dilatados en músculos y corazón (respuesta para luchar o escapar). Otra de las acciones de las catecolaminas es la hiperglucemia y el aumento de los ácidos grasos en sangre. Durante esta fase, todos los cambios hormonales y químicos son acumulativos y relacionados con la intensidad del episodio. Si el estímulo estresante perdura o la intensidad aumenta, el animal no será capaz de restablecer su equilibrio u homeostasis y puede sufrir extenuación fisiológica inducida por estrés. En este caso, se agotan las catecolaminas, produciéndose una bajada súbita de la presión arterial y la glucemia. En este momento el animal está muy expuesto a sufrir hipoglucemia, hipertermia, fallo cardíaco, colapso circulatorio, shock

y muerte. Un ejemplo de este caso sería la captura manual forzada y mantenida en el tiempo de un animal salvaje (nutria, lobo, corzo, rebeco) (Nielsen 1999).

En animales salvajes se han descrito dos tipos de estrés: 1) el estrés primario o de efecto inmediato sería aquel al que se somete un animal cuando es perseguido y capturado. 2) el estrés secundario o de efecto a largo plazo sería la respuesta de los animales al transporte y la adaptación a nuevas condiciones (cambio de instalaciones, cautividad, etc.) (Nielsen 1999).

Cada especie animal presenta respuestas físicas específicas frente a los agentes estresantes por lo que el personal que maneja animales salvajes debe estar familiarizado con las diferentes especies con las que trabaja (por ejemplo la paloma faisán *Otediphaps nobilis* puede morir solo al capturarla si no es anestesiada de inmediato; las gazelas dorcas *Gazella dorcas neglecta* se tumban y permanecen quietas en situación de alerta; la nutria *Lutra lutra* responde con un comportamiento muy agresivo, mordiendo cuando intenta ser capturada).

Un síndrome, denominado “miopatía de captura” fue descrito por primera vez en África, durante las capturas del antílope de Hunter (*Damaliscus hunteri*) (Nielsen 1999) y desde entonces se ha descrito en numerosos especies de aves y mamíferos incluyendo a la nutria (Hartup et al. 1999; Williams & Thorne 1999). Este síndrome se caracteriza por la destrucción de los músculos cardíacos y esqueléticos asociada con los desequilibrios fisiológicos producidos por el estrés extremo (captura, persecución, extenuación). Ha sido también referida bajo otras denominaciones: miopatía de estrés, polimiopatía, miopatía del transporte, enfermedad del músculo blanco, paresia espástica, necrosis muscular, distrofia muscular, miopatía degenerativa, miodegeneración, rhabdomiolisis y parálisis de extremidades. La miopatía de captura, estrechamente ligada con el estrés de la captura de animales salvajes, debe ser considerada como una complicación posible y evitable en cualquier proyecto de translocación de animales salvajes (Spraker 1994;Williams y Thorne 1999).



Una vez un animal salvaje es capturado y mantenido en cautividad, cesa el estrés primario o inmediato, pero el animal continua expuesto de manera constante a nuevos agentes estresantes, (por ejemplo voces y olores humanos, nuevos alimentos, vallas, ruidos, etc. Las respuestas en esta fase, varían en función de la especie: algunos animales dejan de comer o beber, otros huyen o intentan escapar constantemente, mientras que en otras ocasiones – si las condiciones y los cuidados son los adecuados – los animales pueden acostumbrase rápidamente a los humanos. En caso de no superar esta fase, los animales sufren un desequilibrio energético con pérdida de energía, masa muscular, debilidad, hipoglucemia, shock y muerte.

Entre las principales medidas descritas para prevenir o atenuar los efectos del estrés y evitar la aparición de la miopatía en las capturas de animales salvajes, destacaremos las siguientes (Nielsen 1999; Spraker 1994; Swart 1993):

- Evitar las capturas con temperaturas ambientales extremas.

- Mantener a los animales inmovilizados protegidos de las temperaturas altas o bajas.
- Mantener a los animales inmovilizados el menor tiempo posible.
- Reducir al mínimo el número de personas involucradas en las capturas.
- Evitar juntar especies diferentes, así como animales agresivos dentro de un cercado o separación.
- Seleccionar las drogas anestésicas adecuadas y a la dosis precisa; nunca rebajar dosis de manera sistemática por “miedo”. Los animales parcialmente inmovilizados padecen mucho más estrés ya que son parcialmente conscientes y “luchan” hasta la extenuación para escapar.
- Nunca transportar animales en cajas sin ventilación o en número mayor del adecuado. Protegerlos durante el transporte frente a los agentes externos: lluvia, viento, temperaturas extremas, etc.
- Evitar las persecuciones prolongadas de los animales previamente a la captura; evitar capturar animales “extenuados”. Si después de más de dos minutos de persecución el animal no puede ser capturado, puede ser más juicioso posponer el procedimiento.
- Las cajas o cercados de contención deben ser lo suficientemente altos como para que los animales no intenten escapar. Si los animales ven o intuyen una posible huida no cejarán en el intento lo cual producirá altos niveles de estrés o incluso miopatía de captura.
- Durante las inmovilizaciones mantener al mínimo los estímulos visuales, auditivos, olfativos y táctiles en los animales.
- Posteriormente a las capturas, mantener a los animales en reposo al menos 6 semanas.
- Evitar la captura de animales muy viejos o debilitados.
- Evitar la captura de hembras preñadas o lactantes.

Se han llevado a cabo numerosos estudios con el fin de “cuantificar” o medir la respuestas de estrés en diversas especies animales con especial énfasis en su captura y mantenimiento en cautividad (Harlow et al. 1987; Palme et al. 2000; Palme & Möstl 1997; Parrot et al. 1994; Salpolsky 1994; Hatting 1993; Hatting et al. 1988; Kock et al. 1987; Marco et al.1997; Morton et al. 1995; Schwarzenberger et al. 2000). Parece obvio que es muy difícil medir de una manera objetiva los agentes estresantes. Hatting (1993) propone la medición de una serie de parámetros con el fin de cuantificar el estrés. Estos son: hematocrito, lactato, glucosa, osmolaridad, cortisol y catecolaminas totales. Morton et al (1995) proponen la medición del cortisol en plasma como

un buen indicador del estrés sufrido por 712 animales capturados en Africa. Read et al. (2000) en un estudio para valorar el estrés en wapiti (*Cervus elaphus canadensis*), se basó en la temperatura rectal, frecuencia cardíaca, frecuencia respiratoria, fórmula sanguínea, bioquímica, cortisol sanguíneo, lactato, gases sanguíneos y en el patrón de la actividad de los animales.

Parece aceptado de manera general que los estímulos o agentes estresantes pueden inducir la secreción de la hormona ACTH (hormona adrenocorticotrópica), la cual incrementaría la síntesis y excreción de cortisol en la médula adrenal. Por este motivo, los análisis de cortisol y sus metabolitos en los excrementos han sido empleados con frecuencia como un método para cuantificar el estrés en animales salvajes (Dukelow & Dukelow 1989; Grahan & Brown 1996; Jurke et al. 1997; Palme et al. 2000; Palme et al. 1999; Palme & Möstl 1997; Schwarzenberger et al. 2000; Whitten 1998). La principal ventaja de este método estriba en que no es invasivo ya que la toma de muestras puede ser realizada sin necesidad de manipular a los animales (al contrario de los estudios que requieren de muestras de sangre o saliva). El cortisol, una vez en sangre circulante, es metabolizado y sus metabolitos son excretados vía bilis en las heces pudiendo ser medidos a través de varios test como el ELISA para el 11,17 dioxoandrostano. Así mismo, también pueden medirse el cortisol y la corticosterona.

Además de los cambios fisiológicos y bioquímicos que el estrés induce en los animales, sabemos que el estrés también produce cambios en el comportamiento (intentos de escape, anorexia, estereotipias, incremento o descenso en la actividad, etc.) por lo que otra alternativa para determinar los niveles de estrés en animales salvajes, sería la realización de estudios de observación del comportamiento (Fowler 1995).

6. Uso de neurolepticos

Entre los métodos descritos para evitar o reducir el estrés en animales salvajes se incluye el uso de neurolépticos (Read et al 2000)

Según el psiquiatra Bodemer (1992), en la historia de la psiquiatría se han producido tres hechos revolucionarios: Freud y su escuela en el siglo XIX, el inicio de la farmacoterapia en 1952 (Dely y Deniker) y por último el desarrollo de sistemas más eficientes de administración de fármacos – en este contexto se incluiría el nacimiento de los neurolépticos de larga duración o *depot*.

Los fármacos antipsicoticos, neurolépticos o tranquilizantes, se encuentran entre los medicamentos más empleados en medicina humana. Se trata de fármacos psicotropos –es decir, con efecto sobre las funciones psíquicas- que actúan principalmente sobre el síndrome esquizofrénico, mejorando o suprimiendo la mayoría de sus síntomas primarios y secundarios. Además, controlan las desviaciones de la conducta y mejoran la capacidad de adaptación (Florez et al 1980). El término "tranquilizante" –más empleado en medicina veterinaria- procede del diseño de una silla con correajes de inmovilización a quien su diseñador (Rush, 1810) denominó "*tranquilizer*". Posteriormente se introdujo el término "neuropléjicos" (1952) y más tarde "*atarácticos*" (1955). Actualmente estas sustancias a las que nos referiremos a continuación son denominadas en base a su acción específica (por ejemplo, antisicóticos, antidepresivos, ansiolíticos, etc.) o de manera más genérica como neurolépticos. En general, también se emplea el término "agentes o productos psicofarmacologicos o psicoactivos" para referirse a todas aquellas sustancias capaces de modificar las percepciones, sensaciones, estado de ánimo y la actividad mental y física de los individuos. (Bodemer 1992).

Los neurolepticos o tranquilizantes se suelen clasificar de la siguiente manera (Flores et al 1980; Meltzer & Swan 1992; Pretorius 1992; Ebedes 1993):

TIPO	EJEMPLOS*
Fenotiacinas y derivados	**Acetilpromazina**, clorpromazina, trifluoperazina, thioridazina, **perfenazina**, **propionilpromazina**, **pipotiazina**, **zuclopentixol**
Tioxantenos	Flupentixol, tiotixene, clorprotixeno
Dibenzodiazepinas	Clozapina, **diazepam**
Butirofenonas	**Haloperidol**, **droperidol**, pimozida, **azaperona**
Benzamidas	Sulpiride
Dihidro indolonas	Molindona
Imidazoles	**Xylacina, detomidina, medetomidina**
Dibenzoxacepinas	Loxapina

*Marcados en negrita los agentes más empleados en veterinaria

En medicina humana los neurolépticos se emplean para el tratamiento de los siguientes procesos (Pretorius 1992):

- Psicosis idiopáticas (esquizofrenia, enfermedad esquizofrenoide, enfermedad esquizo afectiva, paranoia, psicosis reactiva, enfermedades afectivas)
- Psicosis secundarias (por ejemplo, secundarias a alguna etiología orgánica identificada)
- Agitación severa o comportamiento agresivo
- Alteraciones en la movilidad: por emplo, enfermedad de Huntington
- Otras condiciones médicas: por ejemplo anti-eméticos

En los proyectos de translocación, los animales son capturados, confinados en cajas de transporte, alojados temporalmente en lugares desconocidos para ellos (cuarentena, áreas de observación o de pre-suelta) y liberados en medios en ocasiones hostiles. Durante estas fases los animales salvajes pueden estar expuestos a los siguientes factores (Ebedes, 1993; Burroughs 1993):

- Ansiedad, miedo y pánico inicial causado por la captura.
- Proximidad a ruidos y olores instintivamente asociados con el peligro.
- Confinamiento en espacios cerrados y pequeños como cajas, reservas, mangas, vehículos, etc. que impiden la huida de los peligros potenciales.
- Proximidad a los seres humanos y a sus instrumentos (vehículos, ropas, aviones, humos, etc.).
- Alojamiento antinatural de un elevado número de individuos en una superficie reducida. Los animales están obligados a mantener estrecho contacto con sus congéneres.
- Exposición a temperaturas extremas y anormales; frío en transportes nocturnos o en invierno y calor cuando se juntan varios animales o en el interior de las cajas o vehículos.
- Ventilación inadecuada durante el transporte.
- Fatiga y agotamiento como consecuencia de la captura.
- Hambre y sed.
- Hipoglucemia y depleción de las reservas energéticas.
- Dolor y heridas infringidas durante el proceso de captura o antes.

Ebedes y Raath (1999) entre otros, apuntan una serie de recomendaciones para prevenir o reducir el estrés durante la manipulación y el transporte de los animales salvajes y entre ellas destacan el uso de tranquilizantes tales como haloperidol, azaperona y diazepam o de neurolépticos de larga duración como el enantato de perfenazina y el acetato de zuclopentixol.

7. Neurolépticos de larga duración o *depot*

Por definición los neurolépticos de larga duración o *depot* (LAN, del inglés: *Long Acting Neuroleptics*) son tranquilizantes o neurolepticos en los que una única aplicación proporciona niveles efectivos terapéuticos durante al menos 7 días (Lingjaerde 1973). La obtención de un efecto tan prolongado se puede realizar mediante cuatro sistemas:

- La liberación lenta desde el punto de inyección.
- La absorción lenta del producto.
- La metabolización lenta de los metabolitos activos del producto.

- La eliminación lenta de los tejidos diana.

Los LAN presentes en el mercado se obtienen por la disolución del producto genérico (por ejemplo la perfenazina) en forma de éster de ácido graso en aceites vegetales o medicinales (como sésamo). Tras la aplicación, se produce una hidrólisis lenta del aceite con liberación del éster. Este difunde desde el solvente hacia el líquido extracelular para posteriormente ser absorbido por el torrente sanguíneo donde es hidrolizado en la forma activa. En este momento el producto hidrolizado en sangre tiene las mismas propiedades que la droga no esterificada que encontramos en las preparaciones ordinarias (no *depot*) (Ebedes 1993).

El primer LAN desarrollado (1965) fue la flufenazina decanoato (Modecate®; Squibb) (Ayd 1975) y todavía hoy día es empleado con frecuencia en psiquiatría humana. En animales salvajes los LAN fueron aplicados por primera vez por Ebedes en Sudáfrica (1984) para intentar contrarrestar parte de los efectos adversos del estrés de captura y manipulación, gracias a su efecto prolongado de sedación y a su capacidad para reducir la ansiedad y la actividad motora. Mediante estos fármacos los animales permanecen relajados y sedados, y aceptan mejor las nuevas situaciones sin parecer totalmente conscientes de ello. Comienzan a comer y a beber antes, pierden interés por pelear y se adaptan mejor a la cautividad.

Los principales signos generalmente observados en animales salvajes tratados con LAN son (Ebedes 1993):

- Modificación de la disposición de los animales hacia el medio y los animales que les rodean.
- Indiferencia hacia el nuevo hábitat.
- Estimulación del apetito y de la ingestión del agua, presumiblemente debido a la falta de miedo.
- Tendencia a tolerar mejor la presencia humana y ausencia de pánico.
- En algunos casos, cuando los animales salvajes pierden el miedo a las personas pueden incluso llegar a atacarlas (por ejemplo kudu *Tragelaphus strepticeros,* blesbock *Damaliscus dorcas,* rebeco *Rupicapra rupicapra*).

Existen el mercado multitud de neurolépticos. En la siguiente tabla se exponen los principales empleados en animales salvajes y de Zoo (varias fuentes):

Nombre genérico	Nombre comercial (presentación)	Dosis (mg/kg) y vía administración	Duración del efecto
Acción corta			
Propionil promazina	Combelen (Bayer)	0.03-0.2 (IM, IV)	4-6 h
Acepromazina	Calmo neosan	0.125-0.25 perros, gatos;0.05-0.1 caballo, oveja, vacuno (IM, IV); 1-3 PO	4-8 h (efecto residual hasta las 12 horas)
Haloperidol	Haloperidol (5 mg/ml)	Dosis muy variables IM, IV (0.11-0.48)	8-12 h
Tioridazina	Meleril	1 (en varias tomas PO)	?
Azaperona	Stresnil (40 mg/ml)	0.5 en herbívoros; 1.0 carnívoros; 1-2 marsupiales	2-3 h
Depot			
Flufenazina (decanoato)	Modecate (25 mg/ml)	12.5-75 mg	21-28 d
Flupentixol (decanoato)		20-200 mg	14-28 d
Haloperidol (decanoato)		1.0-4.5 en marsupiales	7-30 d
Pipotiazina (palmitato)	Lonseren	100-200 mg; 25 en *Thrysomys swinderianus*; 10 marsupiales	21-28 d; <10 d
Perfenazine (enantato)	Trilafon (100 mg/ml)	3.0 (*Acinonyx jubatus*); 0.5-5 en	Hasta 7 d

		marsupiales	
Zuclopentixol (decanoato)	Cisordinol depot (200 mg/ml)	10 marsupiales;	10-21 d; < 10 d
Zuclopentixol (acetato)	Cisordinol-Acuphase (50 mg/ml)	1 (*Cervus elaphus*); 0.6 (*Acinonyx jubatus*)	

8. Perfenazina enantato (Trilafon®)

Entre los LAN más empleados en animales salvajes destaca la perfenazina (enantanto; Trilafon®; Sherring). Se trata de un miembro de las fenotiacinas cuyos principales efectos se resumen en la tabla siguiente y deben ser considerados antes de su aplicación:

EFECTO CENTRAL	EFECTO PERIFÉRICO
Bloqueante multipotencial; antagonismo dopamina; bloqueo alfa 1 adrenergico; antiserotoninérgico; antimuscarínico y antihistaminico:	Bloqueo alfa-1-adrenergico; inhibición de la liberación de catecolaminas:
Sedación Catalepsia y efectos piramidales a altas dosis Potenciación de sedantes y anestésicos Anti emesis Inhibición de la termorregulación Inhibición de secreción hormonas: FSH, LH, MDH, ADH y oxitocina Incremento de hormonas: prolactina	Inotropismo negativo Vasodilatación-hipotensión Inhibición de la eyaculación Potenciación de toxicidad de organofosforados Residuos en leche, carne y huevos.

Su acción no se aprecia hasta transcurridas unas 10-16 horas de su aplicación (intramuscular profunda). El máximo efecto se obtiene a las 72 horas y dura entre 7 y 14 días según los diferentes autores (Ebedes 1993; Blumer 1991). En personas (única especie en la que hemos encontrado estudios farmacocinéticos), posteriormente a la aplicación de 100 mg (1.2-1.6 mg/kg para una persona de 60-70 kilos), se detectan concentraciones de perfenazina (media 0.001 mg/L) en sangre durante 14 días.

Aunque su empleo en animales salvajes está ampliamente documentado (Ebedes y Raath 1998), existen pocos estudios sobre su empleo en mamíferos no artiodactilos (Wintere y Wiesnner 1998; Huber et al. 2001) y no hemos encontrado ninguna referencia sobre trabajos de farmacocinética en animales salvajes o de Zoo. Por este motivo, las pautas y dosis empleados en animales se basan únicamente en estudios de comportamiento. En medicina humana, además de las consultas psiquiátricas, durante las últimas décadas se ha empleado la monitorización de los niveles en plasma de estas drogas para optimizar farmacologicamente los tratamientos. Por otra parte, existen diferencias individuales y de edad en las concentraciones en plasma y su eliminación de la sangre puede ser más rápida que su eliminación de tejidos u órganos con alto contenido lipídico (como el sistema nervios central). Por estos motivos y por la dificultad de la obtención de muestras seriadas en animales salvajes, los estudios farmacocinéticos son difíciles. Sin embargo, creemos importante la realización de estos estudios en especies en las que se prevén futuros usos de estos productos para establecer unas correctas pautas de tratamiento.
Las pocas referencias que hemos encontrado sobre el uso de perfenazina enantato en carnívoros son las siguientes:

Especies	**Dosis (mg/kg)**	**Duración (días)**	**Efecto máximo (día)**	**Referencia**
Guepardo (*Acinonyx jubatus*) (n=6)	3	6 (11-14?)	2	Huber et al. 2001

Oso pardo (*Ursus arctos*) (n=1)	1.6	?	3	Winterer & Wiesner 1998
Oso polar (*Ursus maritimus*)	1.0	5	3	Winterer & Wiesner 1998
Guepardo (*Acinonyx jubatus*) (n=3)	0.5-0.6	?	4	Winterer & Wiesner 1998
Jaguar (*Panthera onca*) (n=2)	0.5	?	4	Winterer & Wiesner 1998
León (*Panthera leo*) (n=4)	0.5	?	5	Winterer & Wiesner 1998
Ocelote (*Felis pardalis*) (n=2)	0.4	?	5	Winterer & Wiesner 1998
Serval (*Felis serval*) (n=1)	0.5	?	3	Winterer & Wiesner 1998
Tigre (*Pantera tigris*) (n=1)	0.5	?	3	Winterer & Wiesner 1998
Pantera negra (*Pantera pardus*) (n=1)	0.5	?	3	Winterer & Wiesner 1998

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OBJETIVOS

Los objetivos de la presente tesis son:

1- Llevar a cabo una amplia revisión de los aspectos veterinarios publicados de los mustélidos, en particular de la nutria (capítulo 1).

2- Establecer los valores hematológicos y bioquímicos de referencia para la nutria ibérica salvaje (capítulo 2).

3- Valorar la eficacia y seguridad de una combinación de medetomidina y ketamina en la nutria ibérica con el fin de establecer un protocolo de anestesia eficaz, seguro y reversible que pueda ser aplicado en futuros planes de manejo de esta especie (capítulo 3).

4- Revisar y analizar las técnicas veterinarias utilizadas durante la captura, manipulación y cuidado en cautividad de las nutrias durante el proyecto de reintroducción de la nutria en Cataluña (PRNC) (1995-2000), (capítulo 4).

5- Evaluar la influencia del estrés en los principales parámetros hematológicos y bioquímicos en nutrias salvajes recién capturadas (capítulo 5).

6- Determinar las variaciones en estos parámetros en función del tiempo transcurrido por los animales en cautividad y del uso de neurolépticos de larga duración (enantato de perfenazina - Trilafon®) (capítulo 5).

7- Valorar la seguridad del uso de enantato de perfenazina (Trilafon®) en la nutria y establecer la farmacocinética de este fármaco en esta especie (capítulo 6).

El capitulo 1 esta basado en

Mustelidae. Jesús Fernández-Moran. Zoo and Wild Animal Medicine 5th Edition. W.D. Saunders, Philadelphia, USA, 2002

CAPITULO 1

Biology and Anatomy

The family Mustelidae includes approximately 63 species of terrestrial carnivores or piscivores inhabiting all continents except Australia, New Guinea, Madagascar, and Antarctica. They have been introduced in New Zealand. In the course of evolution, they have developed several adaptations regarding behavior and many physical features. Some species live mainly in the ground or even partially underground, whereas others are active also above the ground in trees. Some have selected the saltwater or freshwater as their preferred habitat.

Included in this family is the smallest living carnivore, the common weasel; the largest representatives are the giant and sea otters; and on land, the wolverine. Many mustelids have body masses of less than 1 kg, whereas the sea otter may reach 45 kg.

The family Mustelidae includes five subfamilies. The weasel-like carnivores (Mustelinae) represent the group with the greatest number of species, comprising 10 genera with approximately 33 species including weasels (11 species), polecats (3 species), and minks (2 species). The subfamily Mellivorinae is represented by only a single species, the honey badger or ratel (*Mellivora capensis*). Subfamily Melinae includes five genera with eight species of badgers represented in Africa, Australia, South America, and wide ranges of northern Eurasia and North America. Skunks (subfamily Mephitinae) are exclusively common in North America. Otters (subfamily Lutrinae) are small to large forms that show the most highly developed adaptations to marine life of all mustelids. They lead an amphibious life and feed mainly on fish or crustaceans. Most scientists recognize four genera and 13 species.

Most mustelids have a highly flexible spinal column; the limbs are comparatively short, ending in feet with five digits, and they walk digitigrade or plantigrade. The claws are not (or are

only partly) retractable. They present the typical carnivore dentition with teeth number varying from 28 to 38. Developed canine teeth are always present, and the last premolar in the upper jaw and the first molar in the lower jaw jointly form the “crushing shears” for processing food. The dental formula of weasels is I 3/3, C 1/1, P 3/3, M 1/2 on the upper and lower jaws. In the wolverine the formula is I 3/3, C 1/1, P 4/4, M 1/2 upper and lower. The Eurasian badger formula is I 3/3, C 1/1, P 4/4, M 1/2 upper and lower, and in the members of the genus *Lutra* the formula is I 3/3, C 1/1, P 3-4/3, M 1/2 upper and lower. Glands may be located in various regions of the body surface. Particularly characteristic are the paired anal glands that produce odorous secretions characteristic of the species and are used for marking their habitat. Some species can spray these secretions over long distances as a method to discourage or harm enemies.

Mustelids are predominantly solitary. Males and females come together only during the reproductive period, and social communities generally include mother and offspring. Tables 49-1 and 49-2 summarize biological data of selected mustelids.

Unique Aquatic Adaptations

The Mustelidae family contains numerous fully terrestrial species, two that are semiaquatic (minks), and a number that are more aquatic (the Lutrinae). The latter have adaptations for the aquatic habits that can be relevant for the clinical management. Underwater vision presents challenges for the mammalian eye: the need for increased sensitivity to light, accommodation of the spectral shift toward the blue-green wavelengths, and modification of the ocular focusing capacity because of refractive differences compared with those in air. Different adaptations for these challenges have been proposed, although visual acuity in water is somewhat reduced in some otter species (i.e., Oriental small-clawed otter). Little is known of the importance, sensitivity, and mechanisms of hearing by otters in the aquatic or terrestrial environment. Olfaction has been retained as an important sense for aquatic mustelids, largely but not exclusively in support of their activities on land. However, evidence indicates that otters have less complex scent-production capacities than do terrestrial mustelids, and that scent production by sea otters may be more poorly developed and less important than for other otter species.

These changes probably have resulted from the increased importance of vision and the reduced importance of olfaction in the aquatic environment. Insulation in aquatic mustelids is achieved by means of a dense underfur that prevents water penetration to the skin while providing flotation. Because fur is an efficient insulator, furred aquatic mammals require some means of controlling heat flux; for example, in sea otters most heat flux is conducted through the enlarged rear flippers. In otters and mink, swimming is the primary means of locomotion. They demonstrate many adaptations that enhance swimming performance and reduce transport costs while in the water: body streamlining; large, specialized plantar surfaces for propulsion; and the ability to remain submerged for extended periods. However, most otters, unlike most aquatic mammals, are capable of quadrupedal locomotion on land and this is the reason why they are considered morphologically intermediate between terrestrial and aquatic mammals.

Feeding and Nutrition

Within the Mustelidae family is a great variation in food habits. Some are strict carnivores (ferrets, weasels, polecats, etc.), some omnivorous (skunks, badgers, or tayras), and fish eaters (otters) (Tables 49-1 and 49-2). The digestive tract is characterized by a simple stomach and a short gastrointestinal tract with no cecum. The more omnivorous species have flattened molars. Captive mustelid species are fed on a great variety of items: commercial dry dog, mink, and cat food, cereal diets mixed with meat or fish, and fresh or frozen fish, shellfish, crabs, and crayfish. Fruits, vegetables (carrots, lettuce, green beans, cucumber, collard greens, kale, and potatoes, among others), eggs, and live or killed food items (crickets, mealworms, mice, and small mammals) also have been included. Target dietary nutrient values for mustelids are based on several sources. The cat is typically the model species used to establish nutrient guidelines for strict carnivorous animals. The National Research Council (1986) and the Association of American Feed Control Officials (1994) have provided recommendations for cats. A limited amount of information is provided by the NRC publication for mink and foxes, which represents requirements of another mustelid species (Table 49-3). The complete dietary requirements of domestic ferrets are still a matter of some controversy, with no one particular diet currently being recommended. In the ferret and mink the protein in the diet should be of high-quality and easily digestible because of their short gastrointestinal transit time of 3 to 4

hours. Generally, most mustelids need a diet high in good-quality meat protein and fat and low in complex carbohydrates and fiber. High levels of protein from plant sources have been associated with urolithiasis in mustelids and are therefore undesirable. Food should be offered at least twice a day, and water must be available. When developing appropriate dietary management of a specific mustelid species, one must consider the following: feeding ecology, target nutrient values, food items available at zoos, and information collected from diets offered by institutions successfully maintaining and breeding for the species.

Restraint and Handling

Even though some captive mustelids can be gentle with their keepers, and all members of this family may be handled with nets, snares, or squeeze cages, caution must be used while managing wild mustelids because they have needle-sharp teeth, are agile and aggressive, and can inflict severe bites. They are also potential carriers of rabies, so should be handled with caution. Leather gloves should be used by operators when handling any kind of mustelid, no matter the size. The ferret is best restrained when grasped above the shoulders, with one hand gently squeezing the forelimbs together with the thumb under the chin of the animal. Minks are grasped by the tail with one hand, while the other grasps the animal behind the neck with the thumb and finger around the head. Polecats, ermines, weasels, and martens are restrained better initially with a net and then injected manually. Skunks defend themselves by spraying the secretions of the anal sacs, and they may bite as well. The defensive position assumed by a threatened skunk is with hindquarters facing the enemy, feet planted firmly on the ground, and tail straight up in the air. They should be captured with a net from behind a shield of glass or plastic, or the handler should wear goggles and protective rain gear. Larger mustelids like otters, badgers, and wolverines may be placed in a squeeze cage for manual injection of a tranquilizer or may be injected directly by means of a pole syringe or a blowpipe.

Mustelids are susceptible to stress caused by improper handling. Otters, particularly sea otters, are susceptible to stress caused by handling and transporting. Different techniques have been developed for safe management of this species.

Only trained personnel should handle mustelids, and usually a combination of physical and chemical restraint is warranted to reduce stress and the subsequent capture myopathy that may occur. Restraint should be brief, and care should be taken to avoid oral cavity and limb traumas.

Chemical Restraint

Many different drugs have been used extensively for the chemical immobilization of mustelids. In most species, dissociative/benzodiazepine/alpha 2 agonist combinations have been used and are highly recommended for induction or short-term anesthesia. Ketamine can be used alone or with midazolam, diazepam, xylazine, medetomidine, or acepromazine to improve muscle relaxation. Xylazine or medetomidine combined with ketamine has been recommended for several species, and both combinations can be reversed with atipamezole (2.5 to 5 mg per mg medetomidine, and 1 mg per 8 to 12 mg xylazine). Tiletamine-zolazepam is another option. Doses ranging from 2.2 to 22 mg/kg have been reported for numerous species of mustelids. In otters the usage of a low dose of tiletamine-zolazepam to achieve anesthetic induction, then supplementation with isoflurane or ketamine (5 mg/kg) for maintenance, has been advocated. Flumazenil (0.05 to 0.1 mg/kg) maybe used to antagonize the zolazepam portion of this combination to hasten recovery, but its usage has not been reported in mustelids other than the North American river otter. Drugs and dosages commonly used to provide chemical restraint and sedation in selected mustelids are listed in Table 49-4. These combinations usually provide short periods of chemical restraint. If longer periods of anesthesia are needed, gas anesthesia (methoxyflurane, halothane, isoflurane, and sevoflurane) delivered by induction chamber, mask, or endotracheal tube works efficiently, although the results of chamber induction with inhalation agents may vary.

Whenever possible, the following parameters should be recorded when mustelid is immobilized: actual weight, relative oxyhemoglobin saturation (clamp located in tongue, lips, ears, or toes), heart and respiratory rates, and rectal temperature. Possible anesthetic complications than may occur include respiratory depression (apnea, bradypnea, tachypnea, or hypoxemia), hyperthermia, hypothermia, bradycardia, tachycardia, poor myorelaxation, and

excitable recovery. During recovery from anesthesia, animals should be kept in a quiet, dark, den box or confined area to facilitate smooth anesthetic recovery.

Clinical Pathology

Blood may be collected from various sites; the technique and site depend on the species, how much blood is needed, and the operator preference. Sites include the jugular vein, cranial vena cava, ventral coccygeal artery, median caudal vein, lateral saphenous vein, cephalic vein, and femoral vein. Published reference ranges for hematological and serum biochemistry analyses in selected mustelids are listed in Tables 49-5 and 49-6. Techniques for urine collection, urinary catheterization, splenic and bone marrow aspiration, placement of intravenous and intraosseous catheters, administration of fluids, and blood transfusion have been described for the ferret and can be useful when treating other mustelids. A technique of mandibular salivary gland biopsy for rabies testing has been developed in North American river otters. Other diagnostic techniques such as ultrasound, electrocardiography, radiology, and auscultation are applicable but vary for every species.

Diseases

Infectious Diseases

The following viral diseases have been reported in mustelids: Aleutian mink disease (plasmacytosis), influenza, canine distemper, rabies, rotavirus diarrhea, infectious canine hepatitis, pseudorabies (Aujeszky's disease), transmissible mink encephalopathy, mink enteritis, epizootic catarrhal enteritis of ferret (possibly coronavirus) feline panleukopenia, canine parvovirus, feline leukemia, Powassan virus disease (arbovirus), and herpes necrotizing encephalitis (herpes simplex).

The following bacteria have been identified as pathogenic in mustelids: *Helicobacter mustelae, Desulfovibrio* spp., *Campylobacter jejuni, C. coli, Salmonella* spp., *Clostridium perfringens* type A, *C. botulinum, C. welchii, Mycobacterium* spp., *Actinomyces* spp.,

Pseudomonas aeruginosa, P. putrefaciens, Streptococcus spp., *Staphylococcus* spp., *Erysipelothrix rhusiopathiae, Escherichia coli, Klebsiella pneumoniae, K. ozaenae, Bordetella bronchiseptica, Listeria monocytogenes, Yersinia pestis, Y. ruckeri, Bacillus anthracis, Brucella abortus, Pasteurella multocida, P. pseudotuberculosis, Francisella tularensis, Leptospira* spp., *Bacteroides melaninogenicus, Proteus vulgaris, P. mirabilis,* and *Plesiomonas shigelloids.*

Fungal diseases rarely are reported in mustelids, but those cited include histoplasmosis, cryptococcosis, blastomycosis, coccidiomycosis, mucormycosis (*Absidia corymbifera*), adiaspiromycosis (*Emmonsia crescens*), and dermatomycosis (*Microsporum* sp. and *Trichophyto*n sp.).

Table 49-7 contains data of the most common infectious diseases reported in mustelids.

Parasitic Diseases

Although not generally associated with disease, numerous external and internal parasites have been identified in wild and captive mustelids. Table 49-8 includes data regarding some selected parasites reported to produce disease in mustelids. Parasitic diseases could be more important for wild animals undergoing translocation projects because of the immune suppression possibly induced by stress.

Ectoparasites

External parasites reported to affect mustelids include the following: fleas (*Ctenocephalides canis, C. felis, Pulex irritans, Nosopsyllus fasciatus, Ceratophyllus gallinae, Chaetopsylla globiceps, Parceras melis, Spilopsyllus cuniculi,* and *Monopsyllus sciurorum*), ticks (*Ixodes ricinus, I. bansksi, Amblyomma americanum,* and *Dermacentor variabilis*), lice (Orders Mallophaga and Anoplura), demodectic mange (*Demodex* sp.), sarcoptic mange (*Sarcoptes scabiei*), ear mites (*Otodectes cynotis*), myiasis (*Cuterebra* spp. and *Wohlfahrtia vigil*), guinea worm (*Dracunculus insignis*), and filarial dermatitis (*Filaria taxidae*). Mite, tick, and flea treatments include the concurrent treatment of the environment and animals. On

animals, compounds that are approved for use in cats are recommended (rotenone or pyrethrin powders and sprays). Organophosphates and carbamates should be used with caution, because safe levels for mustelids have not been established.

Endoparasites

Protozoal infections include *Giardia* spp., *Isospora* spp., *Eimeria* spp., *Sarcocystis* spp., *Toxoplasma gondii, Neospora caninum, Sarcosporidium* sp., *Besnoitia* spp., *Hepatozoon* spp., *Pneumocystis carinii, Trypanosoma cruzi,* and *Cryptosporidium* spp.

Although a variety of helminths are reported in mustelids, few helminth parasites are found in zoo and wild mustelids. These helminths include the following: lung flukes (*Paragonimus westermani* and *P. kellicotti*), intestinal flukes (*Nanophyetus salmincola* and *Troglotrema acutum*), liver flukes (*Fasciola hepatica*), Acanthocephala (*Corynosoma semerme, C. strumosum,* and *Macrocanthorhynchus ingens*), tapeworms (*Taenia* sp., *Monordotaenia* sp., and *Oschmarenia* sp.), *Trichinella* sp., lungworms (*Skrjabingylus* spp., *Crenosoma* spp., *Perostrongylus* spp., and *Filaroides* spp.), heartworms (*Dirofilaria* spp.), ascarids (*Ascaris* spp., *Baylisascaris devosi,* and *Toxocara canis*), *Dioctophyme renale, Dracunculus* spp., *Strongyloides* spp., *Capillaria hepatica, Uncinaria* sp., *Euyhelmis squamula, Aonthotheca putorii, Eucoleus* sp., *Pearsonema plica, Molineus patens,* and *Mastophorus muris*.

Table 49-9 lists the most commonly used drugs and doses for controlling infectious and parasitic diseases in mustelids.

Noninfectious Diseases

The following conditions have been reported to affect wild and domestic mustelids (Table 49-10).

Nutritional Diseases

Renal calculi (calcium oxalate and urate calculi) were detected in 66.1% of the captive North American adult population of Asian small-clawed otters that had been radiographed or necropsied, and prevalence in wild-born otters was 76.7%. The captive diet appears to be a contributing factor to urolith formation and progression. Other medical problems associated with nutrition in mustelids are hypovitaminosis A; vitamin E, thiamin (Chastek's disease), calcium, zinc, and biotin deficiencies; zinc toxicity; rickets; nutritional secondary hyperparathyroidism; fibrous osteodystrophy; gastric trichobezoars; dental disease (dental tartar, gingivitis, and periodontal disease); gastric and duodenal ulceration; and gastric bloat.

Metabolic Diseases

Urolithiasis (magnesium ammonium phosphate, calcium oxalate, calcium urate, calcium phosphate, and ammonium urate uroliths), hypocalcemia, pregnancy toxemia, agalactia, hyperestrogenism, hormonal alopecia, idiopathic hypersplenism, gastric dilation (possibly associated with *Clostridium welchii*), dental and skeletal anomalies, periodontal disease, amyloidosis, hyperadrenocorticism, insulinoma, diabetes mellitus, fatty liver, cardiovascular calcification, osteomalacia, and degenerative joint disease have been reported.

Neoplasms

More than 50 different neoplasms have been reported in domestic ferrets. Although no current consensus exists on the cause of the high incidence of neoplasms, several theories have been proposed: genetic predisposition, early neutering of ferrets at 5 to 6 weeks of age, lack of natural photoperiod or exposure to natural sunlight, diet, and infectious agents. However, neoplasms in species other than ferrets are not common and include seminoma, leiomyoma, adenocarcinoma, pheochromocytoma, teratoma, lymphosarcoma, anal sac carcinoma, lymphoreticular tumor, bronchoalveolar carcinoma, malignant melanoma, and a tumor resembling Hodgkin's disease.

Miscellaneous Diseases

Reproductive toxicity from polychlorinated biphenyls and polychlorinated dibenzo-*p*-dioxins, organophosphate and carbamate intoxication, mortality associated with melanorsine, petroleum residue exposition, mercury toxicity, secondary exposure to rodenticide, shock, exertional myopathy (capture myopathy), trauma, intestinal volvulus, pneumoperitoneum, uterine torsion, interspecies aggression, behavior problems, cystic kidneys, dilative cardiomyopathy, cor pulmonale, intervertebral disk disease, tail alopecia syndrome, overgrowth of claws, oral foreign bodies, intestinal ulcers, pyometra, capture-related injuries (mostly digit and tooth damage), pulmonary silicosis, fibrocartilaginous emboli, and trauma (mostly shooting, vehicle encounters, and trapping) have been reported.

Reproduction

Important variations exist in the reproductive cycles of different mustelids. Some data for representative species are listed in Table 49-11. Most mustelids are seasonal breeders, although the sea otter and the Eurasian otter are exceptions. The duration of the breeding season can vary from 1 month (African striped weasel) to 12 months (Eurasian badger). Some mustelids are polyestrous and others are monoestrous. The duration of estrous periods range from 3 to 5 days to 5 to 8 weeks. Most males that have been studied have active spermatogenesis for only about 3 to 4 months out of the year, although exceptions such as the Eurasian badger may occur. Mustelids can be induced or spontaneous ovulators.

Many mustelids are known to have delayed implantation, including the sea otter, American river otter, hog badger, American and Eurasian badgers, ratel, striped skunk, western spotted skunk, wolverine, all martens, ermine, long-tailed weasel, mink, and marbled polecat. In those species, embryo development proceeds to the blastocyst stage and then ceases. This period of blastocyst dormancy is called diapause and varies from few weeks in mink and striped skunk to almost a year in the Eurasian badger. Extensive studies have been conducted on the mechanisms that control embryonic diapause in three species of mustelids: mink (*Mustela vison*), Eurasian badger (*Meles meles*), and western spotted skunk (*Spilogale gracilis*). Numerous investigators have speculated on the ecological significance and selective pressures that might have favored development of delayed implantation. Changes in photoperiods are known to alter

the secretion of pituitary hormones and thus the onset and duration of breeding, puberty, and timing of implantation. In this way, photomanipulation has been used in some species. Adequate numbers of animals should be maintained to ensure mating, but compatibility does not ensure reproductive success. If copulation or gestation does not occur, different pairings should be tried; but in some cases, animals that are not compatible during most of the year often will breed if introduced during estrous. For this, determining when females are in estrous may be crucial. Different methods have been proposed in different species including behavioral changes, vulvar swelling, vaginal cytological examination, and fecal and urinary hormone analysis. In males the testes enlarge during breeding season. Pregnancy can be determined by urinary progesterone and conjugated estrogen levels, palpation, radiography (end of gestation period), and ultrasonography.

In ferrets, continued high levels of estradiol resulting from persistent estrus can lead to alopecia and bone marrow suppression, resulting in pancytopenia and even death, so nonbreeding females should be neutered.

No specific recommendations are made for mustelids, and ovariohysterectomy, vasectomy, and castration are currently the safest permanent sterilization procedures of birth control.Melengestrol acetate hormone implants have been used successfully for female mustelid contraception. These implants should be removed after 2 years for one pregnancy if possible, and are not recommended for more than a total of 4 years. The human contraceptive implant known as Norplant, which contains the synthetic progestin levonorgestrel, has been used to prevent pregnancy in striped skunks. Depo-Provera injection (5 mg/kg every 2 months) also is indicated. Although no data exist for mustelids, progestin contraceptives may be associated with progressive uterine growth resulting in infertility, infections, and sometimes uterine cancer.

Preventive Medicine

Periodic examinations should include the following:

- Checking transponders and tattoos and reapplying them if necessary
- Checking baseline physiological parameters (weight and breeding status)

■ Examining the oral cavity
■ Evaluating the reproductive tract
■ Taking radiographs
■ Collecting blood for hematological and biochemical examination
■ Checking for heartworm in endemic areas using a heartworm enzyme-linked immunosorbent assay antigen test
■ Banking serum
■ Performing fecal examination for internal parasites (and administering anthelmintics if necessary)
■ Updating vaccinations

Table 49-9 list some of the antiparasitic drugs commonly used in mustelid medicine. Other drugs (e.g., antibiotics) are dosed at rates for the dog and cat.

The following vaccinations are recommended. Few viral diseases have been reported in mustelids other than the ferret, although they have been vaccinated routinely against a variety of viral diseases. Mustelids have varying susceptibility (species- and exposure-dependent) to feline panleukopenia, canine distemper, rabies, and leptospirosis. Most authors recommend vaccination of mustelids for rabies and canine distemper. Safety and efficacy of modified live canine distemper vaccinations in exotic species of carnivores has been problematic because vaccine-induced distemper has occurred (e.g., a modified live virus vaccine derived from chick embryo cell culture killed four female black-footed ferrets [*Mustela nigripes*] or protection was not achieved). In the past, killed distemper vaccines have not provided long-standing protection in most species. Recently, a recombinant canarypox-vectored canine distemper virus vaccine (Merial, Ltd., Inc., Athens, Ga.) has been found safe and efficacious, and if commercially available in a monovalent form, it appears to be the best choice for general mustelid protection against canine distemper virus. If a canine distemper modified live vaccine is used, it should be given separately and not in multiple forms because immunosuppression and other untoward vaccine interactions might lead to disease. Ferret or mink cell culture–derived modified live virus vaccines should never be used in mustelids. A modified live canine distemper vaccine of primate kidney tissue cell origin, Onderstepoort type (Galaxy D, Schering-Plough Animal Health

Corporation, Omaha, Neb.) has been proved to be safe and efficacious in hybrid black-footed ferret–Siberian polecat. The only vaccine approved by the U.S. Department of Agriculture for ferrets is Fervac-D (United Vaccines, Madison, Wis.), an egg-adapted strain that has induced anaphylaxis in some mustelids, so its use is not recommended.

Vaccination schedules for nondomestic species are based on studies of the domestic dog. Neonates receiving colostrum should be vaccinated every 3 to 4 weeks between 6 and 16 weeks of age. Colostrum-deprived neonates should be given two vaccinations administered on a 3- to 4-week interval and starting at 2 weeks of age because maternal antibodies acquired in utero may be absent by 4 to 6 weeks of age. Data on maternal antibody interference with vaccination of ferrets suggest that a final canine distemper virus vaccine should be administered after 10 weeks of age.

The author recommends that veterinarians consider the risk of contracting the disease against the risk of vaccine-induced distemper when designing a preventive medicine plan at each institution. If an animal has an adverse reaction, the veterinarian should be ready to administer an antihistamine (e.g., diphenhydramine hydrochloride, 0.5 to 2 mg/kg intravenously or intramuscularly) or, for severe reactions, epinephrine (20 μg/kg intravenously, intramuscularly, subcutaneously, or intratracheally) and to administer supportive care.

Mustelids also are vaccinated with a killed rabies vaccine, although the efficacy of these vaccines has not been proved in exotic mustelids. Rabies should be given at 16 weeks for animals at risk of contracting rabies, whereas adults should be vaccinated annually.

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Table 49-1. Biological Information of Selected Species of Mustelids

Scientific Name	Common Name	Mass*	Geographical Distribution	Distinguishing Features	Life Span	Food
Mustela nivalis	Common weasel or least weasel	F, 30-120 g M, 36-250 g	North Africa, western Europe, eastern Siberia, Japan, Alaska, and northeastern United States (New Zealand)	Smallest species of family; body size and fur color highly varied	About 1 year	Burrowing voles, true mice, birds, frogs, and lizards
Mustela erminea	Ermine, stoat, or short-tailed weasel	F, 85-200 g M, 200-310 g	Europe to eastern Siberia, Japan, and Alaska to northern Greenland to northern United States (New Zealand)	Summer fur cinnamon-brown or even yellow on back; underside white	About 1 year	Burrowing voles, true mice, hares, birds, eggs, lizards, and frogs
Mustela putorius	European polecat	F, 650-820 g M, 1000-1500 g	Europe	Probable ancestor of domestic ferret, *M putorius furo;* facial mask	5-6 years; 10 years or more in isolated cases	Small rodents, rabbits, hares, birds, eggs, frogs, snakes, and insects
Mustela nigripes	Black-footed ferret	F, 750-850 g M, 900-1000 g	Alberta to northern Texas	Facial mask; black limbs	12 years	Prairie dogs and other small rodents and birds
Mustela lutreola	European mink	400-1200 g	Western Siberia and	Polecat-like; long vibrissae	7-10	Mouselike rodents,

			eastern Europe (western Europe)	on snout	years	fishes, crayfish, mollusks, birds, amphibians, and reptiles
Mustela vison	American mink	500-2300 g	Canada, United States, (Iceland, north and central Europe, and Siberia)	Sparse white spots on chin and ventral side; otherwise, similar to European mink	8-10 years	Same as European mink
Vormela peregusna	Marbled polecat	370-715 g	Southeastern Europe to western China	Spotted back; large ears	8 years	Gerbils, jumping mice, susliks, hamsters, and other rodents
Poecilogale albinucha	White-naped weasel	F, 230-290 g M, 280-380 g	South Africa to Zaire and Uganda	White neck; stripes on back	5 years	Small rodents, birds, snakes, grasshoppers, and other insects
Ictonyx striatus	Zorilla or African striped polecat	420-1400 g	Senegal, Ethiopia, and South Africa	Stripes on back; squirts secretion from anal glands for defense	13 years	Small rodents, birds, eggs, and insects
Martes martes	Pine marten	F, 800-1300 g M, 1200-1600 g	Western Europe to western Siberia	Summer fur thin and short; winter fur thick and long	15 years	Mouselike rodents, squirrels, hares, rabbits, birds, eggs, reptiles, amphibians, insects, fruits, berries, and nuts
Martes foina	Stone marten or	F, 1100-1500 g	Western Europe to the	Similar to pine marten but	Unknown	Similar to pine marten

	beach marten	M, 1700-2400 g	Himalayas and Altai	heavier, shorter limbs, and white throat spot		
Eira barbara	Tayra	4-6 kg	Northeastern Mexico to Argentina	Dark brown to black body	18 years	Guinea pigs, harelike rodents, birds, reptiles, insects, honey, and fruits
Mellivora capensis	Ratel	7-13 kg	Northern India to Arabia, Africa, and southern Sahara	Some animals completely black; forelimbs muscular with strong claws	Unknown	Small rodents, birds, eggs, lizards, snakes, turtles, frogs, insects, honey, berries, fruits, and roots
Meles meles	Badger	7-13 kg, summer; 15-25 kg, fall	Europe, Japan, and southern China	Silvery gray back and flanks; throat, chest, belly and legs black or brown	16 years	Mouselike rodents, small birds, eggs, frogs, lizards, insects, snails, earthworms, fruits, nuts, and berries
Taxidea taxus	American badger	6-8 kg, summer; 8-12 kg, fall	Southwestern Canada and central Mexico	Thick, dense fur; predominantly gray black with white stripe from nose to root of tail; dark, oblong cheek spot	16 years	Small mammals, birds, eggs, reptiles, insects, and invertebrates
Mephitis mephitis	Stripped skunk	1.2-2.5 kg in the fall up to	Southern Canada to northern Mexico	Black, with mostly two white lateral stripes; sprays	10 years	Small rodents, birds, eggs, insects, worms,

		5.3 kg		secretion from anal glands up to 6 m with accurate aim into eyes of attacker		fruits, berries, and corn
Lutra lutra	Eurasian river otter	5-12 kg	Eurasia, North Africa, Sri Lanka, Taiwan, Sumatra, and Java	Shiny dark brown or chestnut brown back; fingers and toes joined by swimming membranes	22 years	Fishes, crustaceans, clams, frogs, small rodents, and worms
Lutra canadensis	North American river otter	—	Canada and United States	—	—	—
Pteronura brasiliensis	Giant otter	22-32 kg	Venezuela to Argentina	Dark fur; chin, throat, and chest have cream-colored spots; flattened tail; swimming membranes	13 years	Fishes, crustaceans, and other aquatic animals
Enhydra lutris	Sea otter	—	Bering Sea to California	—	—	—

F, female; *M*, male.

brackets refers to areas where the particular species have been introduced

Table 49-3. Reference Nutrient Requirements and Target Nutrient Ranges for the Small-Clawed Otter

Nutrient	Cat[*]	Dog[†]	Mink[‡] (*Mustela vison*)	Artic Fox[§] (*Vulpes vulpes*)	Asian Small-Clawed Otter[‖] (*Aonyx cinerea*)
Protein (%)	24	22	38 (23.9)	24.7	24-32.5
Fat (%)	—	5	—	—	15-30
Vitamin A (IU/g)	3.3	5.0	5.93	2.44	3.3-10
Vitamin D (IU/g)	0.5	0.5	—	—	0.5-1.0
Vitamin E (mg/kg)	30	50	27	—	30-120
Thiamin (mg/kg)	5.0	1.0	1.3	1.0	1-5
Riboflavin (mg/kg)	4.0	2.2	1.6	3.7	3.7-4.0
Pantothenic acid (mg/kg)	5.0	10.0	8.0	7.4	5-7.4
Niacin (mg/kg)	40.0	11.4	20.0	9.6	9.6-40
Pyridoxine (mg/kg)	4.0	1.0	1.6	1.8	1.8-4
Folacin (mg/kg)	0.80	0.18	0.5	0.2	0.2-1.3
Biotin (mg/kg)	0.07	0.1	0.12	—	0.07-0.08
Vitamin B_{12} (mg/kg)	—	0.022	—	—	0.02-0.025
Calcium (%)	0.8	1.1	0.40 (0.3)	0.6	0.6-0.8
Phosphorus (%)	0.6	0.9	0.40 (0.3)	0.6	0.6
Potassium (%)	0.4	0.6	—	—	0.2-0.4
Sodium (%)	0.05	—	—	—	0.04-0.6
Magnesium (%)	0.04	0.04	—	—	0.04-0.07

Iron (mg/kg)	80	60	—	—	80-114
Zinc (mg/kg)	50	50	—	—	50-94
Copper (mg/kg)	5.0	7.3	—	—	5.0-6.25
Iodine (mg/kg)	0.35	1.54	—	—	1.4-4.0
Selenium (mg/kg)	0.1	0.11	—	—	—

[*] National Research Council 1986. Nutrient Requirements of cats. National Academy Press, Washington, DC.

[†] National Research Council 1974. Nutrient Requirements of dogs. National Academy Press, Washington DC.

[‡] Growing and weaning to 13 weeks. Numbers between parentheses are for maintenance. (From National Research Council 1982. Nutrient Requirements of Mink and Foxes. National Academy Press, Washington DC).

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Table 49-4. Drugs and Dosages Recommended for Immobilization of Selected Mustelids

Species	Recommended Anesthetic Combination (mg/kg)	Comments/Alternative (mg/kg)
American badger	Tiletamine-zolazepam (4.4)	Ketamine (15) or xylazine (1)
American river otter	Ketamine (8-12) + midazolam (0.25-5) or ketamine (3) + medetomidine (0.030) (atipamezole)	Ketamine (10-12) + diazepam (0.3-5) or tiletamine-zolazepam (4) + flumazemil (0.08). Respiratory depression may occur.
Asian small-clawed otter	Ketamine (15-18) + midazolam (0.75-1)	Ketamine (4-5) + medetomidine (0.1-0.12) (atipamezole). Respiratory depression may occur.
Black-footed ferret	Ketamine (3) + medetomidine (0.075) (atipamezole)	Ketamine (15) + diazepam (0.1)
Ermine and weasel	Ketamine (5) + medetomidine (0.1) (atipamezole)	Ketamine (3) or tiletamine-zolazepam (11-22)
Eurasian badger	Ketamine (5-10) + medetomidine (0.05-0.1) (atipamezole) or tiletamine-zolazepam (10)	Ketamine (10-16) + xylazine (2-6) or medetomidine (0.04) + tiletamine-zolazepam (2.5)
Eurasian otter	Ketamine (5) + medetomidine (0.5) (atipamezole)	Ketamine (15) + diazepam (0.5). Respiratory depression may occur.
Ferret	Ketamine (10-30) + xylazine (1-2) or diazepam (1-2) or acepromazine (0.05-0.3)	Tiletamine-zolazepam (22), but recovery time can be prolonged.
Giant otter	Ketamine (8.5-10.6) + xylazine (1.5-2)	Prolonged recovery
Marten	Ketamine (10) + medetomidine (0.2) (atipamezole)	Ketamine (60) + xylazine (12)
Mink	Tiletamine-zolazepam (15) or ketamine (40) + xylazine (1)	Ketamine (5) + medetomidine (0.1) (atipamezole)
Ratel (honey badger)	Tiletamine-zolazepam (2.2)	Ketamine (6) + xylazine (0.5)
Sea otter	Butorphanol (0.5) or oxymorphone (0.3)	Fentanyl (0.3) + azaperone (0.25). Caution: numerous reports describe fatal complications.

Stripped skunk	Tiletamine-zolazepam (10)	Ketamine (15) + acepromazine (0.2)
Tayra	Tiletamine-zolazepam (3.3)	—
Wolverine	Ketamine (5-8) + medetomidine (0.1-0.15)	Ketamine (20) + acepromazine (0.2)

Table 49-5. Reference Ranges for Hematological Parameters of Selected Mustelid Species

Parameter*	North American River Otter	Eurasian Otter	Mink†	Striped Skunk	Ferret	European Polecat‡	Striped Skunk‡
Erythrocytes (× 10^6/μl)	6.10-14.50	5.2-7.8	8.07 ± 0.67	6.8-12.2	6.35-11.2	8.39 ± 1.86	8.08 ± 0.68
Packed cell volume (%)	32.2-60.8	37.8-69.1	45.9 ± 3.1	42-61	36.7-54.9	43.6 ± 8.7	43.0 ± 6.5
Hemoglobin (g/dl)	10.4-19.0	11.0-19.9	15. 6 ± 1.1	15-18	11.1-17.1	14.3 ± 2.7	13.4 ± 1.1
MCV§ (fl)	38.3-49.0	60.7-105.2	56.9 ± 1.9	—	45.6-54.7	52.1 ± 407	53.0 ± 2.6
MCH (pg)	11.3-15.8	16.3-26.9	—	—	14.0-17.6	17.3 ± 1.2	17.0 ± 0.4
MCHC (%)	27.8-39.2	24.6-30.9	34.0 ± 0.52	—	30.7-32.9	33.2 ± 1.9	31.8 ± 1.2
Leukocytes (× 10^3/μl)	4.7-33.2	3.1-19.2	6.49 ± 2.02	4-0-19	2.0-9.8	6.20 ± 2.36	8.01 ± 3.12
Neutrophils (× 10^3/μl)	3.0-28.2	1.41-12.86	2.64 ± 1.27	—	0.62-3.33	2.88 ± 1.63	4.22 ± 2.43
Band neutrophils (× 10^3/μl)	0-0.48	0-1.8	0.008 ± 0.020	—	—	0.09 ± 0.05	0.22 ± 0.38
Lymphocytes (× 10^3/μl)	0.12-4.95	0.58-3.84	3.12± 1.05	—	0.83-13.0	2.98 ± 1.73	3.08 ± 1.65
Eosinophils (× 10^3/μl)	0-1.83	0-1.39	0.47 ± 0.44	—	0.13-0.56	0.24 ± 0.19	0.18 ± 0.08
Monocytes (× 10^3/μl)	0-2.38	0-0.99	0.19± 0.13	—	0.18-0.90	0.15 ± 0.11	0.16 ± 0.07
Basophils (× 10^3/μl)	0-0.21	0-0.18	0.05 ± 0.54	—	0.01-0.10	0.10 ± 0.07	0.0 ± 0.0
Platelets (× 10^3/μl)	298-931	178-777	729.58 ± 125.40	—	277-882	303 ± 133	437 ± 0.0
Reticulocytes (%)	—	—	2.1 ± 0.9	—	1-12	—	—

[*] Values are presented as a range or mean plus-or-minus standard deviation.

[†] Values for mink refer to males, although no statistical differences were determined between male and female minks (Weiss DJ, Wustemberg W, Bucci TJ, Perman V. Hematologic and serum chemistry reference values for adult brown mink*J Wildl Dis* 30[4]:599-602, 1994.)

[‡] International Species Information System: Physiological data reference values, ISIS, Apple Valley, Minnesota, USA, 1996.

[§] *MCV,* mean corpuscular volume; *MCH,* mean corpuscular hemoglobin; *MCHC,* mean corpuscular hemoglobin concentratio

Table 49-6. Reference Ranges for Serum Biochemical Parameters for Selected Mustelid Species

Parameter*	North American River Otter	Eurasian Otter	Mink	Striped Skunk†	Ferret	Pine Marten	European Polecat†
Total protein (g/dl)	5.7-9.0	6.0-7.7	5.94 ± 0.31	6.2 ± 1.2	5.1-7.4	6.1 ± 7	5.7 ± 8
Albumin (g/dl)	2.4-4.1	1.25-3.6	2.98 ± 0.14	—	2.6-4.1	3.0 ± 4	3.3 ± 0.4
Globulin (g/dl)	2.9-5.8	2.7-4.8	—	—	—	3.1 ± 4	2.4 ± 0.7
Calcium (mg/dl)	6.8-10.0	5.2-10.3	9.54 ± 0.39	2.43 ± 0.23	8.0-11.8	9.2 ± 1.6	9.12 ± 0.92
Phosphorus (mg/dl)	3.2-8.3	4.2-8.7	5.29 ± 0.79	1.74 ± 0.61	4.0-9.1	4.95± 0.92	6.19 ± 1.70
Sodium (mEq/L)	136-158	142-158	153.7 ± 1.3	149 ± 7	137-162	155 ± 3	152 ± 6
Potassium (mEq/L)	3.5-5.3	3.9-5.7	4.34 ± 0.23	4.8 ± 0.7	4.3-7.7	4.0 ± 0.2	4.7 ± 0.6
Chloride (mEq/L)	94-121	102-125	114.5 ± 1.7	110 ± 6	102-125	126 ± 1	116 ± 8
Creatinine (mg/dl)	0.4-0.8	0.7-1.0	0.71 ± 0.08	1.09 ± 0.80	0.2-0.9	0.79 ± 0.18	0.49 ± 0.20
Urea nitrogen (mg/dl)	17-56	17.3-68.1	15.2 ± 5.6	33.9 ± 32.9	10-45	31.64 ± 11.2	12.5 ± 3.99
Cholesterol (mg/dl)	63-279	95-220	—	172.4 ± 103.8	64-296	176.9 ± 23.0	191.9 ± 52.6
Glucose (mg/dl)	56-225	51-400	125.8 ± 18.7	124.8 ± 62.9	62.5-207	314.5 ± 70.90	106.9 ± 28.9
Serum enzymes	—	—	—	—	—	—	—
Lactic acid dehydrogenase (IU/L)	36-10,820	555-3620	—	581 ± 323	—	1875 ± 520	474 ± 403
Alkaline phosphatase (IU/L)	29-282	9.0-199	71.6 ± 56.9	70 ± 57	9-120	77 ± 29	64 ± 79
Gamma-glutamyltransferase (IU/L)	8-38	—	—	2 ± 3	—	—	10 ± 8
Creatine kinase (IU/L)	67-1300	26-1794	—	895 ± 252	—	555 ± 234	379 ± 384
Alanine aminotransferase (IU/L)	46-990	34-307	—	120 ± 98	82-289	173 ± 44	102 ± 56
Aspartate aminotransferase (IU/L)	34-1260	71-328	67.0 ± 13.7	75 ± 22	28-248	159 ± 18	74 ± 28

* Values are presented a range or mean plus-or-minus standard deviation.

† International Species Information System: Physiological data reference values, ISIS, Apple Valley, Minnesota, USA, 1996.

Table 49-7. Selected Infectious Diseases of Mustelids

Disease	Causative Agent	Epizootiology	Clinical Signs	Diagnosis	Management	Species Reported
Viral						
Canine distemper	Canine distemper virus (Paramyxoviridae)	Transmission of the virus is accomplished most commonly by aerosol exposure or direct contact with conjuntival and nasal exudates, urine, feces, and skin.	Weight loss, anorexia, hyperemia of the face and ears, hyperkeratosis of the nasal planum and footpads, and oculonasal discharge	Histopathological exam; immunofluorescent antibody test on conjuntival smear	Vaccination with a modified-lived canine distemper vaccine of chick tissue cell origin; Onderstepoort-type recommended. Use caution because vaccine-induced distemper may occur.	Domestic ferret, black-footed ferret, American and Eurasian badgers, weasel, striped skunk, Eurasian and American minks, sable, stone and pine martens, polecat, weasel, and Eurasian otter
Influenza	Orthomyxoviridae (several strains)	Transmission by inhalation of aerosol droplets	Sneezing, conjunctivitis, unilateral otitis, fever, and photophobia	Clinical signs and presence of antibodies (hemagglutination inhibition test)	Prevention of exposure of susceptible animals to infected individuals (animals or caretakers). Antihistamine,	Domestic ferret and mink

					antivirals, and antibiotics can be used.	
Aleutian disease and plasmacytosis	Parvoviridae	Infected animals can serve as potential source of infection.	Weight loss, hypergammaglobulinemia, reproductive failure, hemorrhagic enteritis, and immune-mediated glomerulonephritis	Hypergammaglobulinemia usually greater than 20% of total serum protein. Immunofluorescent antibody test, counter immunoelectrophoresis test	No vaccine is available.	Typically a disease of farm-raised mink but has been found in feral mink, domestic ferret, and striped skunk.
Ferret kit disease	*Rotavirus*	Affects kits. Can become enzootic in the facility.	Watery diarrhea, anorexia, and lethargy	Negatively stained virus particles identified in fresh feces	Subcutaneous electrolyte solutions and oral antibiotics (spectinomycin, amoxicillin, and trimethoprim-sulfamethoxazole)	Ferret
Bacterial						

Salmonellosis	*Salmonella newport; Styphimurium; Scholerasuis anatum, S. enteritidis, S. kentucky,* and *S. hadar*	*Salmonella* spp. have been isolated in a number of clinically normal animals. Associated with feeding of uncooked meat.	Hemorrhagic enteritis, dehydration, loss of body weight, fever, and lethargy	Fecal culture	Supportive care and antibiotics	Many mustelids
Tuberculosis	*Mycobacterium* spp. *(M. bovis, M avium-intracellulare,* and *M. tuberculosis)*	Usually infected by eating mycobacterium-contaminated meat	Weight loss, enlarged lymph nodes, chronic respirator disease, and mastitis	Direct examination of tissue and culture	Evaluate zoonotic potential in case of treatment.	Mink, ferret, otter, and Eurasian badger
Campylobacteriosis	*Campylobacter jejuni* and *C. coli*	Ferrets may be asymptomatic carriers. Raw meat diets may predispose mink to infection.	Fever, leucocytosis, abortion, and diarrhea	Fecal culture	Antimicrobials (erythromycin)	Ferret and mink
Botulism	Types A, B, C, and E *Clostridium botulinum, C.*	Caused by eating uncooked or contaminated meat	Animals are found dead or with paralysis and	Fecal Gram stain and toxin assay	Prevention and treatment re difficult.	Otter and black-footed ferret

	perfringens type A, and *C. welchii*	Associated with capture stress in wild otters	dyspnea before dying. Enterotoxemia, acute gastric distension, and cyanosis		Aggressive therapy	
Pneumonia	*Pseudomonas aeruginosa, P. putrefaciens, Streptococcus zooepidemicus, S. pneumoniae, Escherichia coli, Klebsiella pneumoniae, Bordetella bronchiseptica,* and *Listeria monocytogenes*	Concurrent infection with calicivirus or picornavirus may predispose animal to infection.	Labored breathing, dyspnea, cyanotic mucous membranes, increased lung sounds, nasal discharge, fever, lethargy, and anorexia.	Clinical signs, complete blood count results (leukocytosis), culture, and cytological findings	Supportive care and antimicrobial therapy according to test results. Antibiotics to consider include trimethoprim-sulfamethoxazole and cephalosporins	Most mustelids
Anthrax	*Bacillus anthracis*		Acute death with blood draining from body cavities	Staining smears of peripheral blood and *post mortem* lesions	Penicillin-streptomycin	Eurasian badger, honey badger, and mink

Fungal						
Dermatomycosis	*Microsporum* sp. and *Trichophyton* sp.	Transmitted by direct contact or via fomites and is associated with overcrowding and exposure to cats	Skin and hair lesions similar to those reported in other species	Clinical signs are suspicious but diagnosis is made on the basis of a mycotic culture.	Topical treatment with keratolytic shampoos, povidone-iodine scrubs, and antifungal medications	Most species

Table 49-8. Selected Parasitic Diseases of Mustelids

Parasite	Location in Host	Clinical Signs	Diagnosis	Management	Species Reported
Toxoplasma gondii	Multiple organs (disseminated)	Elevated rectal temperature, lymphadenitis, splenomegaly, myocarditis, pneumonitis, hepatitis, and encephalitis	Serological	Prevention. Avoid contact with feline species and feline feces. Treat with pyrimethamine and sulfamerazine.	Skunk, ferret, weasel, polecat, and otter
Lung worms (*Crenosoma* spp., *Perostrongylus* spp., *Filaroides* spp., and *Skrjabingylus* spp.)	Lung and sinus	Cachexia, anemia, coughing, dyspnea, depression, nasal discharge, and neurological signs	Finding the first stage infective larvae in fecal samples	Use of appropiate antihelminthic drug (ivermectine, fenbendazol, or mebendazol)	Mink, skunk, sable, Eurasian badger, otter, and ermine
Kidney worm (*Dioctophyme renale*)	Kidney (usually right kidney)	Weight loss, hematuria, polyuria, renal colic, and trembling	Finding of characteristic ova in urine; radiographs	Surgical treatment (removal of the parasitized kidney); fluid and antibiotic therapy	Mink, otter, weasel, ermine, marten, fisher, and grison
Sarcoptic mange (*Sarcoptes scabiei*)	Skin (specially head and neck)	Scabs form around head and neck, tail, and feet; in advances cases the entire body may be involved.	Finding the mites in skin scraping or biopsy; diagnostic treatment with ivermectin	Ivermectin (0.3-0.4 mg/kg) as a single injection, or 0.2 mg/kg orally every other day for 2 weeks	Most mustelids

Fleas (most often *Ctenocephalides* sp.)	—	May be asymptomatic, pruritus and flea allergy dermatitis, with chronic scratching and rubbing. Severe infestation may lead to debilitation by exsanguination.	Visualization of fleas or flee defecations	Affected animals and enclosures should be treated repeatedly with suitable insecticides (propoxur, Alugan, pyrethrins)	Most mustelids

Table 49-10. Selected Noninfectious Diseases of Mustelids

Disease	Cause	Signs	Management	Prevention	Species Reported
Exertional myopathy	Often associated with recently immobilized, captured, and transported wild animals	Vary with species. Elevated body temperature, depression, lack of response to the environment, ataxia, weakness, dark-colored urine, and elevated renal and muscular serum enzymes	Treatment is rarely successful. Selenium/vitamin E preparations given intramuscularly, balanced electrolyte solution, and prednisolone	Improve methods of capture or restraint. Reduce stress and hyperthermia during animal handling	Badger, otter, black footed ferret
Urolithiasis	Magnesium ammonium phosphate, calcium oxalate, calcium urate, calcium phosphate, and ammonium urate. Primary cause is unknown.	Normally unnoticeable. Abdominal radiographs are the most important diagnostic tool. Signs may be similar to those in dogs and cats.	Treatment is not known.	—	Mink, ferret, Eurasian otter. Small-clawed otter is particularly susceptible.
Petroleum pollution	Spilled petroleum oils (crude or fuel)	Animals look wet and chilled. Lethargy, dermatitis, conjunctivitis, respiratory distress, dehydration,	Primarily symptomatic. Warm intravenous, intraosseous, subcutaneous, isotonic	—	Any aquatic mustelid can be affected.

		malnutrition, anemia, thermoregulatory dysfunction, diarrhea, and neurological abnormalities	fluids, glucose, antibiotics, and glucocorticoids. Good ventilation, flushing the eyes. Hand- or tube-feeding may be required. Monitor blood parameters.		
Polychlorinated biphenyls (PCBs)	Accumulation of high level of PCBs, especially by fish-eating species	Anorexia, bloody stools, fatty liver, kidney degeneration, and gastric ulcers. Population declines; reproductive complications and kit mortality	—	—	Effects diagnosed in mink, Eurasian otter, and polecat. Can affect any piscivorous species.
Amyloidosis	Deposition of amyloid deposit (17 different proteins) locally or systemically	Relate to the specific sites of amyloid deposition. Histological evaluation of tissues obtained by biopsy or necropsy	Usually progressive. Treatment unsuccessful. In human beings, some trials include antibiotics, colchicine, and dimethyl sulfoxide	—	Beach marten, pine marten, mink, wolverine, and Asian small-clawed otter
Thiamine deficency	Thiaminase present in some fish (especially carp, bullhead, smelt, and herring)	Anorexia, salivation, ataxia, incoordination, pupillary dilation, and sluggish reflexes	Parenteral thiamine administration	Supplement with thiamine in piscivorous species.	Mink. Can be a problem in piscivorous species.

Table 49-11. Some Reproductive Characteristics of Selected Mustelids

Parameter	Badger (American, Eurasian)	Ferret, Black-Footed Ferret	Marten (Pine, Stone)	Mink (American, Eurasian)	Otter (North American River, Eurasian)	Giant Otter	Skunk (Striped, Spotted)	Tayra	European Polecat	Common Weasel, Ermine	Wolverine
Gestation	8 months; 9-12 months	41-42 days; 42-43 days	9 months	40-70 days; 35-72 days	245-365 days; 61-63 days	65-70 days	In South: 59-77 days; 50-65 days; in North 230-250 days	63-70 days	40-42 days	34-37 days; 9-10 months	7-9 months
Delayed implantation	Yes	No	Yes	Yes	Yes; No	No	Yes; No, Yes	No	No	No; Yes	Yes
Litter size	1-7; 1-6	1-18; 1-6	2-5; 2-7	3-10; 2-7	2-5; 2-4	1-5	2-10; 2-9	2	4-6	4-7; 4-8	2-3
Mass at birth	90-98 g; 75-85 g	8-10 g; unknown	30 g	6-12 g; unknown	—; 100-120 g	170-230 g	32-35 g; 22 g	75-95 g	7-12 g	0.9-2.3 g; 2.6-4.2 g	80-100 g
Weaning	3 months	6-8 weeks; unknown	4 months	3 months	—; 3-4 months	3-4 months	2 months	Unknown	1 month	60 days; unknown	3 months

Sexual maturity	1 year	4-8 months; in first year	28 months	In first year	23-27 months; in 2-3 years	Unknown	10 months; in first year	1.5-2 year	In first year	115-1150 days; unknown	In 2-3 years
Type estrus*	M; P	P; M	M; —	P; —	M; P	—	M; P	P		—; M	P
Teats (pairs)	4; 3	—	2	4	—; 2-3	—	5-7; 5	—	3-5	5; 4-5	2

* *M*, monoestrous; *P*, polyestrous.

Table 49-9. Parasiticides Recommended for Mustelids

Generic Name	Dosage (mg/kg)	Route of Administration	Comments
Amprolium	50 for 5 days	Oral	Coccidia
Carbaryl (0.5%) shampoo	—	Weekly for 3 weeks	Mange
Dichlorvos	15 for 2 days	Oral	Antiparasitic; organic phosphate
Fenbendazol	50 for 3-5 days	Oral	Alternatively, 20 mg/kg for 5 days
Fipronil	—	Topical	Ectoparasites
Ivermectin	0.2-0.5; repeat every 2 weeks if needed	Subcutaneous or oral	0.006 mg/kg orally monthly for heartworm prevention. Ecto- and endoparasites
Levamisol	10	Oral or subcutaneous	May be toxic at higher dosages.
Mebendazol	15-30 for 3-5 days	Oral	—
Metronidazole	15-20 every 12 hours for 2 weeks	Oral	Protozoa
Nitrofurazone	50 for 10 days	Oral	Coccidia
Praziquantel	5-20; repeat in 2 weeks	Oral or subcutaneous	Cestodes and trematodes
Propoxur	—	Topical	Ectoparasites

Pyrantel emboate	10-60	Oral	—
Pyrethroids	—	—	Ectoparasites
Sulfadimethoxine	30-50 every 12-24 hours	Oral	Antiparasitic, antimicrobial (coccidian)
Thiacetarsemide	2.2 every 12 hours for 2 days	Intravenous	Heartworm adulticide; follow 3-4 weeks later with ivermectin. Mortality has been associated with the use of melarsomine in red panda and North American river otters

El capitulo 2 esta basado en

Hematological and biochemical reference intervals for wild caught Eurasian otter from Spain. J. Fernandez-Moran, l. Molina, G. Flamme, D. Saavedra, and W. Manteca-Vilanova. Journal of Wildlife Diseases, 37: 159-163, 2001

CAPITULO 2

HEMATOLOGICAL AND BIOCHEMICAL REFERENCE INTERVALS FOR WILD CAUGHT EURASIAN OTTERS FROM SPAIN

INTRODUCTION

The Eurasian otter (*Lutra lutra lutra*) is one of 13 species of the family Lutrinae. Although its distribution is larger than that of any other species of otter (Kruuk, 1995), the Eurasian otter has disappeared from many parts of this range, including most or all of England, France, Germany, Holland, Belgium, Denmark, Sweden, Switzerland and Italy (Foster-Turley et al., 1990). In Spain, the Eurasian otter still thrives in the western half of the country, whereas in the eastern part most populations have been severely decimated (Delibes, 1990). A translocation program is currently underway to strengthen the eastern populations with animals from the western part of the country. There has been only one study published on hematological and serum biochemical intervals (Lewis et al., 1998) for the Eurasian otter. However, this study used a variety of different anaesthetic regimes and laboratory techniques which could have increased data variability. Besides, there are some biochemical data not meassured in that study that can be important tools for health assessment of otters. Further, it would be interesting to know whether the normal values obtained from a population of otters from Scotland are applicable to animals from other parts of the species' distribution range. The purpose of this study is to provide reference intervals for hematology and serum biochemistry of wild-caught otters in Spain using the same anaesthetic procedure and laboratory techniques.

MATERIAL AND METHODS

Thirty three Eurasian otters, (11 males and 22 females) were live-trapped in southwestern (Extremadura) (39º30'N; 6º30'W) and northern (Asturias) (43º30'N; 6º30'W) Spain in a period between November 1995 and May 1998. All the animals included in this study were older than 1

year of age, although their precise age could not be determined. Victor double long spring traps (Woodstream Corp., Lititz, Pennsylvania, USA) were placed at night and recovered in the morning using previously described methods (Serfass, 1996).

Trapped animals were chemically immobilized by manual injection of a mixture of 5 mg/Kg of ketamine (Imalgene 1.000, Rhône Merieux, Lyon, France) and 50 µg/kg of medetomidine (Domtor, Orion Corporation, Espoo, Finland) intramuscularly. Physical examinations, including weighing and measuring were performed in all animals. Otters showing signs of illness were discharged and not included in the reintroduction plan. After shipment to the Barcelona Zoo (Barcelona, Spain), they were individually housed indoors in wire- mesh cages (2.44 m long x 1.22 m. wide x 1.22 m. high), with attached wooden nest boxes (0.91 m long x 0.61 m wide x 0.51 m high) and suspended above the ground. Food and water were offered *ad libitum*. The diet consisted of a mixture of fresh trout, chicks, and river crabs.

Otters remained at the Barcelona Zoo during a period between 20 and 30 days in which they were clinically evaluated. Before being released into the wild, they were immobilized using a combination of 5 mg/Kg of ketamine and 50 µg/Kg of medetomidine delivered by blow pipe (Dan-inject, International GmbH, Gelsekirchen, Germany) intramuscularly. Blood was collected after a minimum of a 5 hr fast and time between injection and blood collection varied from 5 to 10 min. Handling included drawing blood from the jugular vein and weighing. Each animal was given a thorough physical examination and individuals showing signs of clinical illness (e.g., depression, anorexia, diarrhea, hyperthermia, infected wounds, weight loss) were not included in this study. Animals were positioned in dorsal recumbency and 10 ml of blood were obtained from the jugular vein using a 20 gauge needle. Seven ml of blood was collected into Vacutainer (Becton-Dickinson, Rutherford, New Jersey, USA) tubes for preparation of serum and 3 ml into tubes coated with ethylene diamine tetracetic acid (EDTA) for hematology. The blood collected for serum chemistry determinations was allowed to clot at 20 C and then centrifuged. The serum was separated and kept at 4 C until analyses. Samples that were lipemic, hicteric, or hemolized were discharged and removed from the study in order to avoid analytical interferences. The samples reached the laboratory 3 to 5 hr after collection and were processed immediately upon arrival. Each otter was monitored during the anesthesia for pulse rate, respiration rate, oxygen

saturation (N-20P, Nellcor, Inc., Hayward, California, USA) and rectal temperature. Thereafter, anesthesia was reversed with atipemazole (Antisedan, Orion Corporation, Espoo, Finland), at a dose rate of five times the initial dose of medetomidine, administered intramuscularly at least 30 min after the induction.

The following hematological parameters were measured using a NE 9000 Sysmex counter (Toa Medical Electronics Corporation, Kobe, Japan): red blood cell count (RBC), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet and leukocyte count. Two blood smears were stained with May-Grunwald Giemsa (Merk, Darmstadt, Germany) and one was examined for the presence of parasites. A leukocyte differential count was performed on the other slide on 100 cells.

Biochemical profiles were measured on a Hitachi 747 automatiated analyzer (Roche Diagnostics Corporation, Indianapolis, Indiana, USA). These profiles included the following parameters: concentration of glucose, total and direct bilirubin, blood urea nitrogen (BUN), uric acid, calcium, iron, triglycerides, cholesterol, total protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, alkaline phosphatase (ALK PHOS), lactate dehydrogenase (LDH), creatine kinase (CK) and alpha-amylase, using Randox reagents (Randox Laboratories, Antrim, UK). Sodium, potassium and chloride were measured with an ion-selective electrode using reagents from Roche (Roche Diagnostics Corporation, Indianapolis, Indiana, USA). Protein fractions, albumin, alpha, beta, gamma-globulin and albumin/globulin, were determinated by capillary electrophoresis using a Paragon CZE tm 2000 with manufacturer's reagents (Beckman Instruments Incorporation, Brea, California, USA).

Serum cortisol concentrations were measured by radio-immunoassay using a GammaCoat M competitive-binding RIA kit (Incstar Corporation, Still Water, Minnesota, USA).

A Kolmogorov-Smirnoff non-parametric test was used to assess the normality of data distributions. Whenever a normal distribution could be assumed, data were summarized by the mean, standard deviation (SD), and minimum and maximum values. When a normal distribution

could not be assumed, the median, inter-quartile Range (difference between 75^{th} and 25^{th} percentiles), minimum and maximum values were used.

A Student's t-test was used to test the null hypothesis of no difference in means by sex. When a variable did not fit normal distribution the Mann-Whitney U test was used (Sokal and Rohlf, 1981).

RESULTS AND DISCUSSION

The results for 19 hematology parameters and 28 serum chemistry values for 33 Eurasian otters were shown in tables 1 and 2, respectively. The data represent healthy animals of both sexes, except for moderate capture stress. Parasites were not detected in blood smears from any animal.

Most of mean values presented are in agreement with those previously reported for the Eurasian otter (Lewis et al., 1998). However, there are some interesting differences. First, we found higher WBC and neutrophil counts as well as lower eosinophil and lymphocyte counts than Lewis et al. (1998). It is possible that these differences are due to stress (Meyer et al., 1992) and suggests that the animals in our study were more stressed by the darting procedure than those in the study by Lewis et al. (1998). Indeed, our blood samples were obtained about 20 days after the animals had been captured in the wild; whereas, in the study by Lewis et al. (1998) the animals had been at a rehabilitation center for at least several months and could have become more accustomed to humans. Indeed, some studies have found differences in the leukograms of several species of carnivores depending on whether the animals had been captured in the wild or kept in captivity (e.g. Fuller et al., 1985; Beltran et al., 1991).

Our platelet counts were lower than those reported by Lewis et al. (1998) . However, it is likely that these differences are due to age; indeed, Lewis et al. (1998) found that juveniles had a much higher platelet count than adults. When only the results from animals older than 1 year of age are considered, their results are similar to ours.

AST and CK activities were higher in our study, a further difference between our study and that by Lewis et al. (1998).This difference could be caused by the fact that our animals, in contrast to those in the study by Lewis et al. (1998), were trapped about 20 days before blood collection. Damage of muscle tissue can be caused by the animals' attempts to escape from a trap and has been shown to increase AST and CK activities (Seal et al., 1975). Nevertheless, since both AST and CK have relatively short plasma lives (Kramer, 1989), this seems unlikely. The higher CK values observed in our study could be caused in part by contamination of blood during venipuncture with intracellular fluid from skeletal muscle. Indeed, Puncture of the jugular vein frequently requires repeated probing with the needle in subcutis, which would contaminate the sample with CK from skeletal muscle (MacWilliams and Thomas, 1992). On the other hand, Lewis et. al. (1998) did not mention in their study the reagents used for these enzymes determinations and it is well known that this can considerably affect the analytical results.

Finally, both cholesterol and BUN concentrations were different as compared with those obtained by Lewis et al. (1998). This may be caused by differences in diet (Williams and Pulley, 1983; Ruiz-Olmo and Palazon, 1997).

Our results confirm that the Eurasian otter has lower red cell counts, but higher MCV and MCH values than the North American river otter (*Lutra canadensis*) (Lewis et al., 1998). Since both species seem to have similar patterns of foraging and diving behavior, these erythrocyte differences are difficult to explain.

Statistically significant differences were observed between males and females in platelets (= 523.68 and 411.45 respectively; P=0.015) and albumin (=3.2 and 2.9 respectively; P=0.004) All these differences do not appear to be clinically relevant. No significant differences were observed between males and females for the rest of the parameters. Consequently, results were combined for the entire sample of 33 otters. Lewis et al. (1998) also found very minor differences between both sexes as in our study. Hematology and serum biochemistry values are similar between males and females in other species of otter (Todcilowski et al., 1997).

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Table 1. Descriptive statistics for 14 hematologic values from 33 wild caught Eurasian otters.

Hematological parameters	Number	Mean	SD	Min	Max
White blood cells (x 10^3/ml)	33	7.32	4.01	3.1	19.2
Red blood cells (x 10^6/ml)	33	6.4	0.66	5.2	7.8
Hemoglobin (g/dl)	33	15.1	2.0	11.0	19.9
Hematocrit (%)	32	54.6	6.9	37.8	69.1
Mean cell volume (fl)	33	85.2	9.4	60.7	105.2
Mean corpuscular hemoglobin (pg)	33	23.6	2.4	16.3	26.9
Mean cell hemoglobin concentration (g/dl)	33	27.7	1.27	24.6	30.9
Segmented neutrophils (x 10^3/ml)	33	4.89	3.12	1.41	12.86
Band neutrophils (x 10^3/ml)	33	<0.1	<0.1	0.0	1.8
Lymphocytes (x 10^3/ml)	33	1.46	0.77	0.58	3.84
Monocytes (x 10^3/ml)	33	0.36	0.26	0.03	0.99
Eosinophils (x 10^3/ml)	33	0.39	0.31	0.0	1.39
Basophils (x10^3/ ml)[a]	33	0.0	0.0	0	0.18
Platelets (x 10^3/ml)	33	486.2	127.7	178.0	777.0

[a] Values outside normality test range. Median, IQR and maximum and minimum values are included.

Table 2. Descriptive statistics for 28 serum chemistry variables from 33 wild caught Eurasian otters.

Biochemical parameters	Number	Mean	SD	Min	Max
Glucose (mg/dl)[a]	33	101	110	51	400
Blood urea nitrogen (mg/dL)	33	33	10.9	17.3	68.1
Creatinine (mg/dl)	33	0.8	0.08	0.7	1.0
Uric acid (mg/dl)	33	2.3	1.1	0.7	5.3
Calcium (mg/dl)	33	9.0	0.9	5.2	10.3
Phosphorus (P) (mg/dl)	33	6.9	0.9	4.2	8.7
Sodium (Na) (mEq/L)	21	152.1	3.7	142	158
Chloride (Cl) (mEq/L)	21	115.1	6.2	102	125
Potassium (K) (mEq/L)	20	5.0	0.4	3.9	5.7
Iron (Fe) (mcg/dl)	33	125.9	93.2	15.0	540.0
Cholesterol (mg/dl)	33	144	27.6	95	220
Total protein (g/dl)	33	6.8	0.4	6.0	7.7
Albumin (g/dl)	32	3.1	0.3	12.5	3.6
Globulins (g/dl)	32	3.7	0.5	2.7	4.8
Globulin Alpha-1 (g/dl)	32	0.2	0.1	0.05	0.7
Globulin Alpha-2 (g/dl)	32	0.9	0.1	0.4	1.2
Globulin Beta (g/dl)	32	1.0	0.1	0.7	1.7
Globulin Gamma (g/dl)	32	1.6	0.5	0.3	2.6
Albumin: globulins (ratio)	32	0.85	0.1	0.5	1.2
AST (IU/L)	33	165.6	68.3	71	328
ALT (IU/L)	33	89.8	58.9	34	307
Total bilirubin (mg/dl)[a]	33	0.14	0.05	0.03	0.9
Direct bilirubin (mg/dl)[a]	32	0.03	0.007	0.01	0.1
Alkaline phosphatase (IU/L)	33	58.6	37.2	9.0	199.0
LDH (IU/L)	33	1599.5	705.1	555.0	3620
CK (UI/L)	33	689.1	390.1	26.0	1.794
Alpha-amylase (UI/L)	32	3.5	4.6	0.0	19.0
Cortisol (mg/dl)	31	1.82	1.73	0.25	8.32

[a] Values outside normality test range. Median, IQR and maximum and minimum values are included.

El capitulo 3 esta basado en

Reversible immobilization of eurasian otters with a combination of ketamine and medetomidine. J.Fernández-Morán, E. Perez, M. Sanmartin, D. Saavedra, X. Manteca-Vilanova. Journal of Wildlife Diseases, 37: 561-565, 2002

CAPITULO 3

REVERSIBLE IMMOBILIZATION OF EURASIAN OTTERS WITH A COMBINATION OF KETAMINE AND MEDETOMIDINE

INTRODUCTION

The Eurasian otter (*Lutra lutra*) is distributed through Europe, Asia and North of Africa. Its population is considered threatened and a total of 4.000 individuals has been estimated for Spain (Ruiz-Olmo and Delives, 1999). Translocation projects are being conducted in different countries including Spain (Sjöånsen, 1997; Saavedra and Sargatal, 1998).

References on immobilization for Eurasian otters are scarce, (Holmes, 1974; Jenkins and Gorman, 1981; Kuiken, 1988; Arnemo, 1990; Vogt, 1994). However, some studies have been published with the similar North American river otter (*Lutra canadensis*).

In this paper, authors report their experiences with numerous immobilizations carried out during a reintriduction project with the aim of determining the efficacy and safety of the combination of medetomidine-ketamine in order to establish an adequate and reversible immobilization protocol in the Eurasian otter for use during translocation projects.

MATERIAL AND METHODS

Thirty eight otters (13 males and 25 females) were live-trapped in South-Western (Extremadura) (39º30'N, 6º30'W) and Northern (Asturias) (43º30'N, 6º30'W) Spain between February 1996 and October 1998. Victor double long spring traps (# 1-1.5 Softcatch, Woodstream Corp., Lititz, Pennsylvania, USA) were set and checked every morning following

the method described elsewhere (Serfass et al., 1996). Otters were urgently transported to the Barcelona Zoo (Barcelona, Spain) by night in an 11 hour trip.

Once the animals arrived at the Barcelona Zoo, they were individually housed indoors in wire-mesh cages (2.44 m long x 1.22 m. wide x 1.22 m. high) suspended above the ground, with attached wooden nest boxes (0.91 m long x 0.61 m wide x 0.51 m high). Food and water was offered *ad libitum*. The diet consisted of a mixture of fresh trout, chicks and crayfish.

Otters were considered adapted to captivity when they started to eat, which usually took about 2-3 days. Animals were released within 30 days of capture, in the Parc Natural Aiguamolls de l'Empordà, (Girona, Cataluña, Spain; 3º05'E, 42º15'N). Throughout the captivity period they were subjected to several medical evaluations including blood sampling, weighing, radiologic studies, and complete physical examination in which anesthesia was required. A total of 82 chemical immobilizations were evaluated.

The anesthetic agents were administered intramuscularly (i.m.) in the hind limb by means of a 2 ml plastic dart equipped with a 1.1 x 38 mm needle and delivered by blow pipe (Dan-inject International, Gelsekirchen, Germany). A mixture of approximately 5 mg/kg of ketamine hydrochloride (100 mg/ml, Imalgene 1000, Rhône Mérieux, Lyon, Francia) and 50 µg/kg of medetomidine hydrochloride (1 mg/ml, Domtor, Orion Corporation, Turku, Finland) was delivered. For reversal atipamezole hydrochloride (5mg/ml, Antisedan, Orion Corporation) was administered i.m. at 5 mg per mg medetomidine hydrochloride. When needed, a complementary dose of ketamine was administered at 2.5 mg/kg. Other products used were intravenous (i.v.) or i.m. atropine sulphate (1mg/ml, Atropina 1 mg, Braun, Barcelona, Spain) at dosage of 0.02 mg/kg when heart rate decreased lower than 100 and oxygen flows administered by nasal tube or facemask at a rate of 2 l per min, in cases when poor oxygenation was detected.

Fasting time was at least 5 hr. During anesthesia animals remained in dorsal recumbency. The eyes were humidified with eye protector drops (Bañoftal, Alconcusí, Laboratorios Cusí, Barcelona, Spain).

The degree and quality of the immobilizations were evaluated as (1) fair: animal was sedated but able to struggle; (2) good: deep sedation but occasional muscle tension or mild struggling when subject to painful procedures and (3) excellent: good muscle relaxation and no response to venipuncture.

Most otters were monitored during the anesthesia for pulse rate and SpO_2 (N-20P pulse oximeter system, Nellcor, Hayward, California, USA) with a D-20 probe placed on the tongue. Respiration rate was determined by breathing movements. Rectal temperature was measured with a rectal thermometer. SpO_2 and body temperature were recorded on 48 events and heart and respiratory rates on 54 and 28 occasions respectively, 15 minutes after darting. On 12 occasions, the heart rate and SpO_2 were recorded at time 15, 20 and 25 minutes after darting.

The following potential problems were considered during the anesthesias: tachycardia; heart rate > 180 beats/ min; bradychardia: heart rate < 100 beats/min; hyperthermia; rectal temperature > 40ºC; hypoxemia; SpO_2 < 80%; movement during the procedure, fail to handling, and poor myorelaxation.

Throughout the immobilizations, behavioral changes were recorded. The initial effect time was defined as the interval between time of injecting and onset of ataxia. Induction time was the interval between the injection time and the time when otters became recumbent and nonresponsive to stimuli. Reversal time was the time from administration of the reversal agent to the time when the animals were able to stand and walk. Analgesia was defined as the lack of purposeful response to a painful stimulus (venipuncture).

RESULTS

In this study 38 Eurasian otters (13 males and 25 females) ranging in body mass from 3 kg to 8.7 kg (mean 5.3 kg) were successfully anaesthetized on 82 occasions. Table 1 summarizes the results of the 82 immobilizations. The induction was rapid and smooth in all cases. Myorelaxation was generally good and according to the quality of the anesthesia, 64 (78%) events were classified as excellent, 17 (21%) as good and only 1 as fair.

Severe bradycardia (less than 70 beats/min) occurred in 4 cases (5%) while moderate bradychardia (heart rates between 70 and 100 beats/min) occurred in 28 cases (34%). Fifty otters (61%) had rates higher than 100 beats/min. Mean value for heart rate increased from 89 beats/min at 15 min to 91 and 97 beats/min at 20 and 25 min respectively. In all the episodes of bradychardia, otters responded within 5 min to atropine (0.02 mg/kg i.v. or i.m.) administration with increasing heart rates.

Rectal temperature data showed only one case of hyperthermia (40.9 C) that was treated successfully with ice packs applied to the body .

Breathing was regular and deep in most cases. Apneas shorter than two min occurred in three animals during the first min of immobilizations. In those cases administration of oxygen maintained the oxygen saturation level above 80% until breathing re-started.

Only one animal had SpO_2 lower than 80%. Mean values for SpO_2 also increased from 91% to 94% and then maintained at 93% (min 15, 20 and 25).

Between 30 and 40 minutes after induction all the animals were given atipamezole at a dose rate of five times of the initial dose of medetomidine i.m. and left in the attached wooden nest boxes without light or external stimuli. Otters recovered gradually and quietly but ataxia was present in most otters at the first stages. In less than 5 min all animals were able to move and responded to external stimuli. Observation period was longer than 5 hr and we observed limb ataxia in a few recovered otters but we never observed resedation.

DISCUSSION

Ketamine has been used in a variety of carnivores alone and combined with xylazine, diazepam, midazolan and medetomidine (Ramsden et al., 1976; Kreeger et al., 1996). Dosages reported for the otter when used alone are as high as 6 to 30 mg/kg (Jenkins and Gorman, 1981; Reuther and Brandes, 1984; Kuiken, 1988; Serfass et al., 1993). In North American river otters, dosages of 10 mg/kg resulted in poor myorelaxation, variable quality of anesthesia,

hyperthermia, struggling and cardiopulmonary complications (Spelman et al., 1993). When combined with the alpha-2-agonist medetomidine the muscle relaxation improves and the anaesthetic depth increases (Spelman et al., 1993) while reversibility is obtained. Ketamine-medetomidine combinations have been used successfully, safely and reversibly in a wide variety of exotic mammals including the otter (Jalanka and Roeken, 1990; Spelman et al., 1994). The EEP/studbook husbandry guidelines for *Lutra lutra* (Vogt, 1994) recommend among others, medetomidine (150 μg/kg) combined with ketamine (5-10 mg/kg) or medetomidine (100μg/kg) with ketamine (5 mg/kg) and midazolan (0.2 mg/kg). Medetomidine (25 μgm/kg) combined with a low dosage of ketamine (2.5 mg/kg) produced stable short-term anesthesia in river otters while severe respiratory depression developed when using ketamine (10 mg/kg) combined with xylazine (1-2 mg/kg) (Spelman, 1999). In a report where 10 Asian otters were successfully immobilized, the author recommended dose rates of 100 to 120 μg/kg medetomidine with 4-5 mg/kg ketamine (Lewis, 1991).

The dosage of 50 μg/kg medetomidine used in this study was based primarily on previous studies with North American river otter (Spelman et al., 1993) and on the author's experience. Lower dosages resulted in an insufficient level of anesthesia whereas higher dosages caused severe respiratory depression (respiration rates bellow 10 respirations/min and SpO_2 lower than 80%). In combination with medetomidine, the immobilizing effects of ketamine are enhanced, allowing a reduction in the amount of ketamine and leading to improved myorelaxation and increased potential for adequate reversal (Jalanka, 1989). We found a dosage of 5 mg/kg ketamine to be effective and adequate for Eurasian otter immobilization. Atipamezole has been reported to cause excitement and overalertness in some wild carnivores treated with medetomidine and medetomidine-ketamine (Jalanka and Roeken, 1990), perhaps as a result of a residual ketamine effect or a high dose. In our study, signs of excitement were of brief duration and in most cases recoveries were smooth and calm.

Hyperthermia has been claimed to be a serious anesthetic complication in otters (Reuther and Brandes, 1984) and has been described as a potential adverse effect of ketamine anesthesia. In our study, however only one case of hyperthermia occurred.

River otters are highly sensitive to the depressant effects of ketamine on the respiratory system (Spelman et al., 1993). Medetomidine in dogs depresses the respiratory rate and may alter respiratory patterns but ketamine tends to ameliorate these effects (Kreeger et al., 1996). In our study respiratory depression did not happen. Otters had in most cases deep and regular breathing. Values for SpO_2 below 90% are considered undesirable and indicate depressed cardiopulmonary function in river otters (Spelman et al., 1997). In our case, mean SpO_2 was higher than 90% (93%). In one case however there was respiratory depression with SpO_2 value as low as 75%. Furthermore, the supplementation of oxygen via facemask or nasal tube is advisable when using this combination. Spelman et al., (1997) recommend to have an endotracheal tube available when injectable anesthetics are used. Under field conditions, reversing the anesthesia and leaving the animal on a quiet and dark pen can be advisable if severe respiratory depression is detected.

Information on baseline heart rate for Eurasian otters is lacking, but Spelman et al., (1993) defined bradychardia in North American river otter as heart rate below 100 beats/min. According to this, bradychardia was a serious concern in this study. Indeed, 39% of otters immobilized with this combination showed heart rates below 100 beats/min. Although the central stimulating effects of ketamine on the cardiovascular system may offset the depressive effects of the alpha 2-adrenergic agonists, significant bradychardia may occur when using medetomidine-ketamine, even at low dosages (Spelman et al., 1994). The advantages of using anticholinergic drugs with alpha 2-adrenergic agonists has not been proven, but concurrent administration of atropine with medetomidine can mediate ventricular arrhythmias in dogs and wolves (Kreeger et al., 1996). In our cases otters responded to atropine (0.02 mg/kg) by increasing heart rates when used. Hypertension may develops with concurrent use of atropine and medetomidine and this effect can be promoted by the dissociative anesthetics (Spelman, 1999). In our case blood pressure was not monitored so we could not determine exactly the importance of the use of atropine. Heart rates increased during the immobilizations in most cases and values fluctuated from 89 beats/min at min 15, to 91 beats/min and 97 beats/min at min 20 and 25 respectively. Values above 180 beats/min which were considered as tachycardia were not seen on this trial.The primary advantage of this combination is potential reversibility. Antagonism with atipamezole was rapid and complete and recoveries were smooth and calm in all the cases shown here.

In conclusion, the anaesthetic protocol studied here consisting of a combination of medetomidine (50 μgm/kg) with ketamine (5 mg/kg) is considered safe and can be recommended in wild caught Eurasian otter for chemical immobilization during translocation projects. It is safe, rapid and can be reversed with atipamezole. However caution is needed as heart depression resulting in bradychardia may occur.

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Table 1. Summary of 82 immobilizations in 38 Eurasian otters (13 males and 25 females) immobilized with medetomidine and ketamine. Pulse rate, respiratory rate, SpO_2 and rectal temperature were recorded at 15 minutes after darting.

Parameters	Number	Mean	SD	Range
Weight (kg)	82	5.3	1.3	3.0-8.7
Ketamine dose (mg/kg)	82	5.1	0.8	3.4-6.6
Medetomidine dose (µg/kg)	82	51	8	34-66
SpO (%)	48	93	6	91-99
Breaths/min	28	32	7	20-44
Heart beats/min)	54	95	21	56-173
First anesthetic effect (min)	82	3	2	2-7
Induction time (min)	82	5.5	3.2	3-9
Body temperature (C)	45	38.4	1.5	31.9-40.9

El capitulo 4 esta basado en

Reintroduction of eurasian otter (*lutra lutra*) in northeastern spain: trapping, handling, and medical management. J. Fernandez-Moran, D. Saavedra, X. manteca-Vilanova. Journal of Zoo and Wildlife Medicine, 33(3): 222- 227, 2002

CAPITULO 4

REINTRODUCTION OF EURASIAN OTTER (*LUTRA LUTRA*) IN NORTHEASTERN SPAIN: TRAPPING, HANDLING, AND MEDICAL MANAGEMENT

INTRODUCTION

Eurasian otters (*Lutra lutra*) disappeared from Girona province (Catalonia, north-eastern Spain) early in the 1980´s due to hunting, pollution, and habitat destruction. Since the possibilities of recolonization from other populations were extremely low[11] and otters have been successful reintroduced in other countries,[8,13,16] a reintroduction project was started in 1993. According to a study carried out in 1994, the Catalonian Otter Project (COP) followed guidelines for reintroductions proposed by the International Union for the Conservation of Nature, [7] with main objectives of restoring the extirpated otter population and promoting river and wetland conservation through support of a "flagship" species.[12]

MATERIALS AND METHODS

Reintroduction area

The reintroduction project was carried out in the Muga and Fluvià river basins, an approximately 2,000 km^2 territory. Both rivers have an irregular water regime and a low absolute volume. The Muga river is 64.7 km long and the Fluvià river is 97.2 km long. Both rivers flow into Aiguamolls de l´Empordá wetlands which includes 4,800 ha protected as Natural Park and 800 ha as a Strict Reserve.[12]

Capture

Fifty five otters were live-trapped with padded leg hold traps (#1-1.5 Soft Catch, Woodstream Corp., Lititz, Pennsylvania 17543, USA) by COP personnel throughout the project from south-western (Extremadura), and northern Spain (Asturias) and Portugal. Captures only occurred in areas with dense otter populations.[11] One factory spring from each trap was replaced by a # 2 spring.[13] Traps were set in water, preferentially in shallow passages between rocks or in river beaches in small tributaries or in main rivers during the summer drought. Traps were bound to 1-m-long chains that were tied to trunks or secured to big rocks using climbing bolts, hammered manually. Traps were set together in groups of two or three withou bait or lures throughout the year when permits were obtained from the appropriate government agencies. May to August months were avoided. “Potential captures” included all captures and escapes, and “capture rate” was defined as the number of otters captured divided by the number of potential captures.[13]

Traps were examined daily between 0500 and 0800. To reduce the risk of injury, otters were chemically immobilized at trap sites with a combination of ketamine hydrochloride (Imalgene 1.000, 100 mg/ml, Rhône Merieux, Lyon 69002, France; 5 mg/kg,i.m.) and medetomidine (Domtor, 1 mg/ml, Pfizer, S.A., Madrid 28002, Spain; 50 μg/kg, i.m.) administered by a blow pipe using plastic darts (Dan-Inject, International GmbH, Gelsenkirchen 45889, Germany).[4] Once it was completely immobilized, each otter was carefully released from the trap, examined, weighed, and its sex was identified.

Otters showing signs of chronic illness or injuries, as well as pregnant or lactating females were immediately released. Young or subadult animals were preferred over older animals. Age was estimated using dentition. Nineteen animals received a dose of the long acting neuroleptic (LAN) perphenazine enanthate (Trilafon enantat, Sheering-Plough S.A.,Madrid 28046, Spain; 100 mg/ml; 20-30 mg, i.m.) to decrease stress level during handling, transport and captive management. After examination, atipamezole (Antisedan, 5 mg/ml; Pfizer, S.A. Madrid, 28002, Spain; 250 μg/kg, i.m.) was manually injected for recovery and otters were placed in transport kennels for recovery in a cold and dark room. In the afternoon, they were transported to the BZ (Barcelona, Spain) by air-conditioned van or commercial airline.

Housing and care a the Barcelona Zoo

Otters were individually housed at 18- 24 ºC in indoor wire-mesh cages (2.44 m long x 1.22 m. wide x 1.22 m. high), with attached wooden nest boxes (0.91 m long x 0.61 m wide x 0.51 m high)suspended above the ground. Alfalfa hay provided bedding material in the wooden nests.

Food and water were offered ad libitum. During the first few days the diet consisted of a mixture of fresh dead or thawed trout, chicks, fresh dead eels, and fresh dead crayfish. After the animals ate normally, their diet consisted mostly of trout. Daily food intake was recorded for every captive animal. Fresh drinking water was provided in a plastic 20 L dishpan. All cages were cleaned and disinfected (CR-36, Laboratorios Collado, S.A., Barcelona 08027, Spain) when their occupants left the zoo.

When otters started to eat, usually 2 d after arriving at the BZ, they were re-immobilized with the same anesthetic regimen for a complete medical evaluation, including radiographs, blood collection,[3] physiological monitoring, implantation of an identifying microchip (Trovan, EID Ibérica S.L., Madrid 20546, Spain), and treatment for any trap-related injuries. Every animal received ivermectin (Ivomec 1%, MSD Agvet. Madrid 28027, Spain; 0.4 mg/kg, s.c.) for the treatment of endoparasites and ectoparasites.

Trap related injuries were classified in four categories: I- no lesions or puncture wounds, lacerations, missing nails, and swelling; II- closed luxation of the interphalangeal joints of one or more digits; III- open luxation of one or more digits; IV- as for III but with exposed or missing phalanges. Oral cavities were examined for tooth damage or wounds produced during capture or transport. Wounds were cleaned, debrided, sutured, and oral antibiotics and nonsteroidal antiinflamatory drugs were administered.

Surgery and release

Several radiotransmitters were used: Advanced Telemetry Systems (470 First Ave, Isanti, Minnesota 55040, USA; 32-40 gm; 30 animals), Telonics (932E, Impala Ave, Mesa,

Arizona 85204, USA; 30 gm; 4 animals) and Wagener (Herwarthstr 22, Köln 50672, Germany; 30 gm; 2 animals). Overall, radiotransmitters were implanted i.p. in 36 otters for monitoring of post release movements and survival. Otters were ready for surgery after 5-10 d in captivity and if severe infections or capture-related diseases had been properly treated or ruled out, and animals had eaten regularly. Surgery was delayed in otters with low food ingestion or signs of disease. Otters were fasted at least 5 hr prior to surgery, but were allowed to access to water.

The same combination of ketamine and medetomidine was injected i.m. by a blow pipe while the otters were inside the wooden nest box. Immobilized otters were intubated with 2.5-3 cuffed endotracheal tubes to establish and maintain inhalation anesthesia with isoflurane (Forane, Abbot Laboratories, Madrid 28027, Spain) and oxygen. Inhalation anesthetics were delivered by a precision vaporizer in an open-circuit system. Otters showing heart rate below 100 beats/min were given atropine (Atropina, 1 mg/ml, Braun Medical, Barcelona 08191, Spain; 0.04 mg/kg, i.m.). An area of 5-6 cm x 4 cm was shaved along the ventral midline over the umbilicus and aseptically prepared with povidone-iodine scrub and 70% ethyl alcohol washes. Each radiotelemetry device was sterilized with ethylene oxide gas and prewarmed (38 ºC) before being placed ventrally in the abdomen following surgical incision (7-8 cm) through the *linea alba.* Three layer closure was done by use of 2-0 polyglycolic acid suture material (Dexon, Braun-Dexon, Barcelona 08191, Spain), in a single interrupted pattern for the *linea alba* and peritoneum, and subcutaneous tissues, and in a discontinuous horizontal mattress pattern for the skin. Following surgery, otters were given a penicillin-streptomycin combination (Dipenisol Retard, Bayer, Bayer, S.A. Barcelona, 08029, Spain; 0.5 ml s.c.) and were placed back into the wooden nest boxes and allowed to recover slowly. Fifteen minutes after extubation a dose of 250 µg/kg i.m. atipamezole was manually injected for recovery. Although drinking water was provided, the plastic dishpans were removed from the cages temporarily for 3-5 days to keep incisions dry during initial healing. Food was provided within 3-5 hr after otters were returned to the cage.

Otters were re-immobilized 10 –12 days after surgery with the same combination of medetomidine and ketamine for clinical evaluation, weighing and radiography to determine the

exact location of the radiotransmitter in the abdomen before release. Otters remained at the BZ for 20 - 30 days.

All otters were transported by car for 2 hr to the release area. Animals that died post release (usually weeks or months later), were returned to the BZ in order to establish the cause of the death and to determine the location of the radiotransmitter.

RESULTS

A total of 8,773 night traps were placed and 55 animals were captured (159 night traps/ otter), with 36 animals escaping (potential captures = 91). The capture rate was 0.60. No otters died during capture. An additional 15 species, including 111 individuals, were accidentally trapped, including the striped-necked terrapin (*Mauremis leprosa*; 32%); mallard (Anas platyrhynchos; 21%); moorhen (*Gallinula chloropus*; 14%); brown rat (*Ratus norvegicus*; 12%), and white stork (*Ciconia ciconia*; 10%).

Forty three captured were transported to BZ. Of these, 79% (n = 34) had category I injuries, 7% (n = 3) had category II, 12% (n = 5) had category III, and 2.% (n = 1) had category IV. No significant oral cavity injuries occurred in most of our animals and only 19% (n = 8) showed lesions attributable to biting or chewing during capture or confinement. Thirty seven percent of the captured animals were male and 63% were female (see table 1). Except for one case, all injuries responded well to treatment and all digit wounds had resolved or were close to complete healing by the time otters were implanted with the radiotransmiter. Otters having an infected wound showed a decrease in body weight even when eating normally.

During transport most otters remained calm, although some tried to escape by biting the steel door or grasping with the front legs. Animals treated with perphenazine appeared to be more calmer and relaxed.

Most otters drank water immediately after being released into the wire cage and shortly afterwards disappeared into the wooden nest box. Otters spent most of their time inside the wooden nests, except at night. Otters appeared to adjust rapidly to captivity and most animals

ate within the first 48 hr post arrival. Most otters ate fresh dead or thawed trout but exceptional animals showed a clear preference for crayfish, chicks, or eels.

Some otters tried to escape during the first two-three nights in captivity. Three males escaped by destroying the joint between wire cage and wood nest, but were recaptured shortly afterwards.

Polyglycolic acid sutures were adequate for the surgeries. Erythema, swelling, or drainage were not observed in any of the 36 animals subjected to surgery; healing of the surgical incisions was rapid and without complications.

Five animals (three males and two females) died in captivity, for an overall mortality rate of 11.2%, with 9% due to management. Otter # 4 died upon arrival at BZ showing signs of respiratory distress. A bronchiolar obliteration caused by a piece of grass possibly aspirated during capture was found on necropsy. Otter #37 never ate, possibly because it was still very young and was severely stressed by the capture process. Otter #5 died 48 hr post arrival due to severe capture myopathy according to clinical and physiologic studies. Otter #43 was injured while trying to escape and suffered a severe infection secondary to a pre-existing pneumonia; this animal never ate. Finally, otter # 15 died after surgery, but before release. On necropsy, a chronic purulent myocarditis presumably caused by a capture-related infected wound on a digit, was found. Neither the animal´s behavior nor its blood values changed, however.

According to field studies, some otters have bred after being released. In 1996 one female still carrying a functional transmitter bred. One cub was observed that year, and two in 1997. In 1999, another female was detected at the den site for 2 mo, but cubs presumably died. The same summer, three sets of cub tracks were observed in the release area. Again in 2000, cub tracks were observed in two different places.

Nine animals died and were retrieved during the year following release. On necropsy, the radiotelemetry devices were free within the ventral abdomen in craniocaudal orientation. There were no mesenteric or omental adhesions, and the surgical incisions had healed completely. Five

otters (56%) were killed by cars, and one each (11% each) by fyke fishing nets, one channel siphons, carbofuran poisoning and unknown causes.

DISCUSSION

Trapping

The breeding cycle of the North American river otter (*Lontra canadensis*) is well known and includes spring breeding, immediate egg development to blastocyst stage, a 9-10 mo delay followed by intrauterine implantation, and a 61-63 day development period of the embryo-fetus before parturition.[10] In contrast, the Eurasian otter is not seasonal and cubs may be born at any time of the year, although births are more common in spring and summer.[11] Consequently, even though most of our animals were captured at other times of the year, juveniles and lactating females were occasionally captured. Although these animals were immediately released, one of the animals that died was a juvenile, but was still included in the project due to its large size and general aspect. This animal never adapted to captivity and rejected food. Translocation of immature individuals should be avoided.

Although our capture method has been previously described,[13] our study involved a greater number of traps per captured otter (60 vs. 159) but with a similar capture rate (0.57 vs. 0.60). In a North Carolina translocation program,[17] trapping success was even higher, with only 26 trap nights per otter. Such differences could be accounted for by variation in the capture areas, as even within our project, trapping success differed considerably between areas.

Blow pipe anesthetic delivery worked well. It avoided causing injury or worsening those produced by trapping, e.g., luxations, fractures, etc. Also, avoiding physical contact with conscious animals, darting them from a distance, and waiting longer than 3 min proved to be safe in our animals, although other authors have recommended nets and manual restraint by trained personnel for anesthesia administration.[14,17] Modified squeeze cages have already been used for otter restraint.[18]

The captured animals suffered very few severe injuries, with only nine animals (21%) suffering some kind of digit luxation. Although comparison with other studies is difficult due to the variety of capture methods used, soft catch traps can humanely capture wild otters.[14] Minor abrasions to foot pads and worn toe-nails on untrapped feet during attempts to pull free of the trap have been reported before.[13]

Unlike previous studies, we observed few dental injuries. [13]. Only eight animals (19%) had this type of lesion Different escape behavior while in traps may be responsible; the escape behavior of our trapped otters consisted primarily of pulling out the trap and digging and destroying surrounding vegetation rather than biting the trap.[13]

Leukocyte count and level of enzymes indicative of muscle necrosis were especially valuable for monitoring clinical improvements of otters suffering from contaminated wounds.[3,14]

Housing

In general captive otters adjusted well to cages and accepted food readily. Live fish (eels and trout) and chicks seemed to act as environmental enrichment tools and elicited eating in some individuals that initially rejected food. Our anesthetic protocol was safe and effective.[4]

The possible benefits of LAN administration during translocation programs of wild caught otters deserves further research, as our observations suggest that they can be beneficial. While there are many studies on their use,[2] data in carnivores is lacking. Although there was a considerable variation in individual behavior, most otters that had received perphenazine appeared much calmer during the 5-7 days post-administration than those that had not, while feeding normally. One animal that had rejected food for 4 days started to eat a few hours after being treated with 20 mg perphenazine enanthate i.m. These animals were less aggressive when the wooden box was opened for inspection purposes and they seemed to react less to external stimuli. Interestingly, treated animals did not kill live chicks, whereas control animals did.

Surgery and breeding

All otters ate normally the day of surgery, showing that this procedure had minor effect on them. The otters did not seem to care about the incision sites and the exposed skin sutures did not become damaged, irritated or infected.

Our radiotelemetry devices appeared to be somewhat too large for intraabdominal use in Eurasian otters, [1, 5] although our 30-40 g devices were considerably smaller than the 110- 120 g devices used in prior studies of North American river otters.[1, 6, 10]

Some authors have advocated immediate surgery and early release in order to reduce stress.[1] However, our animals' good adjustment to captivity, and the risk of incomplete healing after surgery,[1] suggest that post-surgical captivity is convenient. Ten days appears to be an adequate time period.

Some authors have not recommended the ventral midline surgical approach because otters rub their ventrum during grooming. [9,14] Although our otters occasionally rubbed their ventrum, no healing problems occurred. Furthermore, a s.c. and i.p. approach has led to complications.[19] Altough a lateral approach may be an option, [9,14] we recommend the ventral midline approach.

Heat loss from shaving the skin for surgery did not seem to be a problem, perhaps due to the benign climatic conditions in our release area.[6] The final clinical examination confirmed the rapid growth of the inner fur.

Previous studies of radiotransmitters in Eurasian otters concern involved small numbers of animals with inconclusive results. [1,16] Intraperitoneal transmitters have not had detrimental effects on reproduction in North American river otters.[11] Likewise, at least three of our radioimplanted otters later bred successfully after release.[12] The transmitters have also been essential to gathering information about movement and mortality of released animals.

Road traffic accidents were responsible for 83% of otter deaths in a southwest England study and 70% of otter deaths in southern Ireland. [15] These results and ours show that such accidents are the main threat for reintroduced otters in Europe. However, other risks such as poisoning and illegal fish nets exist.

Our approach to capturing, handling, and translocating otters has successfully restored an extirpated otter population in north west Catalonia, Spain and can provide a model for similar programs.

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Table 1. Summary of data on 43 Eurasian otters (Lutra lutra) (16 males, 27 females) handled by the Catalonian Otter Project (COP).

# Animal	Year	Trap Injury Category[a]	Date Captured	Date Released	Days in Captivity	Sex[b]	BWV[c]	Observations
1	1996	I	7/02/96	22/03/96	43	F	U	
2	1996	I	21/09/96	21/10/96	30	F	1,2	
3	1996	III	2/10/96	21/10/96	19	M	0,7	
4	1996	II	2/10/96	3/10/96	1	F	U	Died in Captivity
5	1996	I	10/10/96	11/10/96	1	F	U	Died in Captivity
6	1996	I	9/10/96	28/10/96	19	F	0,6	
7	1996	I	18/10/96	4/11/96	17	M	1,5	
8	1996	I	23/10/96	11/11/96	19	M	0,3	
9	1996	I	26/10/96	11/11/96	16	F	1,5	
10	1996	I	22/11/96	19/03/97	117	M	2,7	
11	1996	I	1/11/96	2/12/96	31	F	0,6	
12	1996	I	1/11/96	2/12/96	31	F	0,5	
13	1996	I	3/11/96	26/11/96	23	M	0,5	
14	1996	I	1/11/96	2/12/96	31	F	-0,4	
15	1996	I	16/11/96	5/12/96	19	M	U	Died in Captivity
16	1996	II	6/03/96	21/03/96	15	F	U	
17	1996	I	20/11/96	11/12/96	21	M	0,3	
18	1996	I	25/11/96	23/12/96	28	F	U	
19	1996	I	25/11/96	13/12/96	18	M	-0,4	
20	1997	I	19/03/97	8/05/97	50	F	0,9	
21	1997	III	4/11/97	10/12/97	36	M	0,8	
22	1997	I	16/11/97	15/12/97	29	F	U	
23	1997	III	16/05/97	21/06/97	36	M	1,2	
24	1998	I	13/05/98	8/06/98	26	M	0,3	
25	1998	II	31/08/98	18/09/98	19	F	0,5	
26	1998	I	31/08/98	18/09/98	19	F	0,5	
27	1998	III	3/09/98	2/10/98	29	F	0,5	
28	1998	I	3/09/98	18/09/98	15	F	0,7	
29	1998	I	29/09/98	15/10/98	16	F	0,2	
30	1998	I	29/09/98	15/10/98	16	F	-0,1	
31	1998	I	30/09/98	15/10/98	16	F	0,1	
32	1998	III	27/09/98	27/10/98	30	F	U	
33	2000	I	19/03/00	11/04/00	23	F	0,8	
34	2000	I	7/03/00	24/03/00	17	M	0,3	
35	2000	I	13/03/00	27/03/00	14	F	1	
36	2000	I	14/03/00	27/03/00	13	M	-0,3	
37	2000	I	14/03/00	19/03/00	5	M	U	Died in Captivity
38	2000	I	26/03/00	14/0/00	19	F	-0,2	
39	2000	I	29/03/00	19/04/00	21	F	0,3	
40	2000	I	24/03/00	19/04/00	26	F	0	
41	2000	I	26/07/00	10/08/00	14	M	-0,2	
42	2000	IV	25/09/00	15/10/00	20	F	0.9	
43	2000	I	28/09/00	12/10/00	14	M	U	Died in Captivity

[a] Trap injury category. I: puncture wounds, lacerations, missing nails or swelling only; II: closed luxation of the interphalangeal joints of one or more digits; III: open luxation of one or more digits; IV: as for III but with exposed or missing phalanges.

[b] Sex; M: male; F: female

[c] BWV: Body weight (kg) variation during captive period. U: unkown.

El capitulo 5 esta basado en

Stress in wild caught eurasian otters: effect of a long acting neuroleptic and time in captivity. J. Fernandez-Moran, D. Saavedra, J.L. Ruiz De La Torre, X. Manteca-Vilanova. Animal Welfare (aceptado para su publicacion Septiembre del 2002)

CAPITULO 5

STRESS IN WILD CAUGHT EURASIAN OTTERS: EFFECT OF A LONG ACTING NEUROLEPTIC AND TIME IN CAPTIVITY

Introduction

Translocation of wild animals is an important tool in wildlife management and conservation. Among others, the Arabian oryx (*Oryx leucoryx*), golden lion tamarin (*Leontopithecus rosalia*), red wolf (*Canis rufus*), black-footed ferret (*Mustela nigripes*), and the river otter (*Lutra canadensis*) have been reintroduced into the wild as part of conservation programs (Clark *et al* 1994; Serfass *et al* 1996)

Capture, handling, transport, and confinement inherent to these projects, inflict a substantial amount of anxiety and fear on animals, particularly when free-ranging wild or semi-wild individuals that have had little previous exposure to humans are to be translocated. Being pursued, caught, and physically manipulated constitutes the most stressful experience ever encountered by the animal. (Nielsen 1999). Some species are particularly susceptible to stress induced by capture and adaptation to captivity situations. This may lead to high levels of anxiety, which in turn may result in refusal of food and water, self-injury, and exhaustion, with fatal consequences. Interestingly, few researches have focussed in animal welfare implications for the individuals to be translocated during such programs and most attention is focused mostly to social implications and spreading of infectious diseases (Seal & Wolf 1992). Exertional myopathy should be one of the most important considerations when planning and executing operations that require handling of wild animals (Williams & Thorne 1996). Also called capture myopathy, has occurred in a wide range of species and appears to be particularly prevalent in primates, birds, and ungulates. The probability of occurrence of this condition may be determined by elevated levels of the intracellular serum enzymes (AST, LDH, and CK) in the blood of the affected animal. (Nielsen 1999). Data from evaluations of serum enzymes may not be of direct management use in the field, but they are useful in later evaluation of the trapping or holding operations (Williams and

Thorne 1996) as this condition may cause the death of the reintroduced animals and therefore compromise the entire project.

There are different techniques to reduce stress related problems during translocation programs. Firstly, reducing or extending time in captivity could result in a reduction of this stress level or in an improvement of the animal general condition, which would improve the survival rate after releasing. In this way, there are controversial opinions about when to release wild caught animals upon capture.

Secondly, using long acting neuroleptics (LANs) is a recently new concept in veterinary medicine that permits an average duration of effect from 1 hour to 28 days depending on the product (Ebedes 1993). In recent years, LANs have been used with increasing frequency in newly caught wild animals to relieve anxiety and facilitate transportation or adaptation (Ebedes 1993**,** Holz & Barnett 1996, McCoy *et al* 1997). The currently used LANs are derived from the phenothiazines or thioxanthenes and depending on the product and the dose given, effects can be maintained up to 30 days LANs have been used primarily for the treatment of human psychotics, especially for the maintenance therapy of acute and chronic schizophrenia. Recently captured wild animals or animals being translocated show alarm symptoms similar to those shown by schizophrenic patients such as anxiety, agitation, psychomotor excitement and aggressiveness that need to be controlled (Ebedes 1993). For veterinary use, the following drugs have been mentioned: perphenazine enanthate, pipothiazine palmitate, fluphenazine decanoate, zuclopenthixol decanoate, flupenthixol decanoate, and zuclopenthixol acetate. Among the LANs available, perphenazine is extensively reported in wild animals during last years. The onset of effect is slow, with sedation and calming effect in wild animals first noted from about 12-16 hr after injection. Maximum effect is usually observed on the third day, with duration of effect being up to seven days (Ebedes, 1993).

Some effects observed in wild antelopes treated with LANs were alteration of mood, indifference to surroundings, and loss of fear to humans. Although the LANs have been used since more than 40 years (Morris & Jarris 1959), most records refer to

ungulates, specially in South Africa and few data were found to any species of carnivore (Winterer & Wiesner 1998). We did not find any reference about using LAN in otters.

The Eurasian otter (*Lutra lutra*) is one of the 13 species of the family *Lutrinae*. Although its distribution range is larger than that of any other species of otter (Kruuk 1995), the Eurasian otter has disappeared from many parts of this range, including most or all of England, France, Germany, Holland, Belgium, Denmark, Sweden, Switzerland and Italy (Foster-Turley *et al* 1990). In Spain, the Eurasian otter still thrives in the western half of the country, whereas in the eastern part most populations have been severely decimated (Delibes & Rodriguez 1990). Some results obtained in the last Spanish otter survey could indicate the recovery of the species in some areas (Ruiz-Olmo & Delibes 1999). For the otter, translocation projects have been carried in different countries (Serfass *et al* 1996, Sjöansen 1997) including Spain (Saavedra & Sargatal 1998), where a re-introduction program is currently underway to strengthen the eastern populations with animals from the western part of the country.

Blood parameters (haematological and biochemical), together with other physiological parameters, are sensitive indicators of alterations in animal homeostasis during capture and stress episodes in wild animals (Kock *et al* 1987, Rietkerk *et al* 1994) and have been proposed as reliable indicators of the stress level during wild animals management (Morton *et al* 1995, Whittington & Grant 1995, Marco *et al* 1997). Some of the blood parameters cited as stress indicators are hematologic values such as hemoglobin, erythrocytes, leukocytes, and biochemical values such as blood urea, albumin, aspartate aminotranferase (AST), alkaline phosphatase (AP), creatine kinase (CK), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), and cortisol (Kock et al 1987, Morton *et al* 1995, Marco *et al* 1997). Different studies have revealed significant differences in these variables in relation to the method used for capturing and handling the animals (Hatting *et al* 1988). The objective of this study was to evaluate the stress influence on some haematological and biochemical parameters in wild caught Eurasian otters during a reintroduction period and to asses its variations related to the use of long acting neuroleptics and the time animals spent under human care.

Material and methods

Twenty eight adult Eurasian otters were live-trapped in South Western (Extremadura; 39º30'N; 6º30'W) and Northern (Asturias; 43º30'N; 6º30'W) Spain between November 1995 and May 1998. Padded leg hold traps (# 1-1.5 Soft Catch, Woodstream Corp., Lititz, Pennsylvania 17543, USA) were placed at night and recovered the following morning as described elsewhere (Serfass *et al* 1996).

Once the animals were located at the trap sites they were chemically immobilised by a manual injection of 5 mg/Kg of ketamine hydrochloride (100 mg/ml, Imalgene 1.000®, Rhône Merieux, 69002 Lyon, France) plus 50 μg/Kg of medetomidine (1 mg/ml, Domtor®, Orion Corporation, Finland) after covering them with a net (Fernandez-Moran *et al* 2001b).

Eleven otters, i.e. the treatment group (TG) were injected i.m. the long acting neuroleptic (LAN) perphenazine enanthate (Trilafon® enantat 100 mg/ml; Schering-Ploug BV, 3606 AN, Maarssen, The Neatherlands) at a dosage of 2.9 - 5.4 mg/kg (average: 4.4 mg/kg).The other 17 otters remained untreated as the control group (CG). All the otters were transported to Barcelona Zoo (BZ) where they were individually housed indoors in wire-mesh cages (2.44 m long x 1.22 m wide x 1.22 m high) suspended above the ground, with attached wooden nest boxes (0.91 m long x 0.61 m wide x 0.51 m high). Food and water was offered ad libitum and the diet consisted of a mixture of fresh or thawed trout, chicks, fresh eels, and crayfish the first 3-5 days and later on only fresh trout. Otters remained at the BZ during a period between 20 and 30 days (average 23 days) in which they were subjected to clinical examinations, quarantine and surgery for intraperitoneal radiotransmitter implantation. No human contact occurred during this period apart from visual inspection during feeding and cleaning time.

All the animals were blood sampled three times during their period in captivity. Animals were considered to have adapted to the new environment when they started to eat, which usually happened between day 2 and 5 post arrival at BZ. At that time they were immobilised and sampled (sample A). When they were considered free of infectious or serious capture related disease, which usually took place 5-10 days post capture, they were immobilised again for intraperitoneal implantation of the radiotransmitter device and blood was obtained again (sample B). animals were allowed

to recover from surgery for a period of between 10 and 12 days . After that, otters were immobilised in order to carry out a post surgery control and releasing them into the wild. At that time, (20-30 days after capture) otters were bled again (sample C). Fasting time was at least 5 hours. After anaesthesia following the methods described elsewhere (Fernandez *et al* 2001b) otters were positioned on their back and 10 ml of blood was obtained from the jugular vein using a 20 gauge needle. Seven ml of blood were deposited into Vacutainer® (Becton-Dickinson, Rutherford, New Jersey, USA) tubes for preparation of serum and 3 ml into tubes coated with ethylene diamine tetracetic acid (EDTA) for haematology. The blood collected for serum chemistry determinations was allowed to clot at 20ºC and them centrifuged and the serum separated and kept at 4º C until the determinations were made. The following haematological parameters were meassured as described by Fernandez-Moran *et al* (2001a): red blood cell count (RBC), haemoglobin (Hb), and white blood cell count (WBC).Biochemical profiles were measured as described by Fernandez-Moran et al., (2001a) and included: blood urea, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, alkaline phosphatase (AP), lactate dehydrogenase (LDH), creatine kinase (CK), albumin, and serum cortisol.

Each otter was monitored during the anaesthesia for pulse, respiration, oxygen saturation (N-20P, Nellcor, Inc., Hayward, California 94545, USA) and rectal temperature. Thereafter, anaesthesia was reversed with atipemazole (Antisedan®, Orion Corporation, Espoo, Finland) administered intramuscularly at least 30 minutes after the induction.

Haematology and biochemical parameters were analysed with repeated measures analysis of variance, using SPSS/PC program (Chicago, USA).

Results

The values of 17 haematological and serum chemistry parameters performed on three occasions for the 28 treated and untreated wild caught Eurasian otters were shown (table 1). Statistically significant difference were observed between perphenazine treated animals and untreated animals only for the monocyte count (means: 0.4 *versus* 1.2; 0.4 *versus* 0.7 and 0.3 *versus* 0.6 10^9/litre for untreated *versus* treated in A, B, and

C samples respectively. $P<0.05$). Consequently, results were combined for the entire sample of 28 otters. However, values for RBC, Hb, WBC, segmented neutrophils, blood urea, ALT, AST, CK, LDH, AP, and albumin were statistically influenced by time in captivity as can be seen in the graphs (Figs 1-11). RBC and Hb, increased over time, while the leukocyte and segmented neutrophil counts decreased. Most biochemical parameters did not changed significantly, but the blood urea, ALT, AST, AP, LDH, and CK decreased significantly overtime. Contrary, albumin increased significantly.

Discussion

We will discuss first the effect of time: two types of stress reactions have been described in newly captured animals. The primary short-term, traumatic stress inflicted on an animal during the act of pursuit, capture, and initial physical manipulation, and the secondary, long term, fatiguing stress imposed on the animal during transport, confinement, and adaptation to captivity (Nielsen 1999). In our otters, it can be difficult to separate both kind of stress response as every time they were manipulated they were stressed somehow although the methodology followed was always the same. The statistical difference observed for RBC and Hb over time is difficult to explain. These two parameters started high to lower in the second sample and finally increased (see figs. 1 and 2). Causes described in domestic animals to produced a reduction of these parameters are anaemia, end of gestation, tranquillisation and anaesthesia, and haemolisis (Bush 1991). Based on this, anaemia could be suggested to have affected our otters during the first days in captivity as a consequence of the capture, transport, and adaptation procedures, than improved over time with the correct management given in captivity. On the other side, we observed higher leukocyte and neutrophil counts in the first samples that were lowering constantly through the study. The effect of stress on the leukocyte count varies with the species and depends upon the normal relative leukocyte distribution. Dogs, cats, and possibly otters, having relatively low lymphocyte counts, respond with an increases in leukocytes (Bush 1991). Leukocytosis and neutrophilia in other carnivores and ungulates have been attributed to capture stress (Kreeger *et al* 1990; Rietkerk *et al* 1994; Weaber & Johnson 1995**)** which would suggest that the stress response decreased with time in captivity in our otters. Also, otters captured may have suffered infected wound or lesions due to capture that would have improved overtime, decreasing thus the leukocyte number.

Evaluations of serum CK is specially useful and although dynamics of CK in serum of wild caught otters have not been determined, high-serum levels seem to reflect active or very recent muscle degeneration and/or myonecrosis. Otters recently captured in this study presented high values of these enzymes suggesting to be highly stressed or proximal to this condition. Stress may induce as a consequence of an increase of protein catabolism, hypoalbuminemia. In our case, the albumin fraction increased during captivity, possibly because of the reduction of the stress level or the better ingestion of food once animals adapted to captivity. Blood urea also decreased while otters remained in captivity but this parameter may be probably related to the diet rather than to the stress condition.

When otters were maintained in quite places, without human contact for many days, these abnormal values for this parameters advocated as stress indicators (high leukocytes, high neutrophils, high AST, ALT, LDH, CK, and low albumin) were gradually reduced and stabilising during the two last samples.

Plasma cortisol level has been extensively used as an stress indicator (Harlow *et al* 1987; Parrot *et al* 1994; Morton *et al* 1995), and its determination altogether with other variables would be the best method for an assessment of stress in wild animals. In our case, no statistical difference was noted concerning cortisol level in blood, probably because this parameter was consistently high because the short term stress response when otters were injected with the dart when manipulated.

There are controversial documentation regarding when to release wild caught animals. The American Society of Mammalogists in its guidelines for the capture, handling, and care of mammals, recommends that translocated animals should be released as soon as possible after capture to minimise behavioural or physiological stresses resulting from the conditions of captivity (ASM 1998). In the same way, Arnemo (1991) elected the idea of immediate surgery and early release to avoid further stress and to minimise the risk of abnormal behaviour when captured 5 wild otters in Norway. However, Hoover (1984) kept all the otters to be reintroduced 5 days postoperatively for daily clinical assessment before released. We followed similar methods described by Serfass *et al* (1996). Although they did not perform stress determination test, they supposed than animals would benefit of being under human

care before being released (Serfass *et al* 1996). Our data presented here, show that this seems to have occurred with our translocated animals. In similar circumstances to our otters, a rest period before releasing can be beneficial for the properly reintroduced animal. Our results show that the wild caught otters released were in better general condition of homeostasis than when recently captured.

Now we will focus on the effect of LAN: in our study no correlation was obtained when comparing haematological and biochemical values of treatment and control group. As mentioned by Ebedes (1993), in wild animals it is impossible to assess, control, and individualise the dosage of tranquillisers and the safest alternative is to use the lowest possible effective dose. We only found reference dosages for wild ungulates ranging from 20-200 mg depending on the size of the animals (Ebedes 1993) and 0.5 to 0.6 mg/kg zoo felids (Winterer & Wiesner 1998). We used a total dose of 2.9-5.4 mg/kg which is relatively high compared with those reported before for other species but Blumer (1991) pointed out that in hoofstock, there appear to be an inverse relationship between dosage of perphenazine enanthate and the average size of the species with larger species requiring lower doses per unit weight. According to our knowledge this is the first time LAN have used in otters so this dosage was elected based on authors previous unpublished experiences.

Although we do not know whether our dosage reached the adequate therapeutic level or not, we did not observe adverse effects such as extrapyramidal symptoms, previously reported with the use of these drugs. Perphenazine enanthate, at the dosage used in this study was ineffective in suppressing the physiological responses to capture stress. However, this does not mean that the administration of this neuroleptic was not beneficial for the otters. They could be approached easily without their becoming alarmed. They were calm and also unresponsive to human presence. This failure of the phenothiazines to reduce the physiological response to capture, while still inducing apparent sedation in undisturbed animals, is consistent with their effects in human patients and has been reported in other species before (Knox *et al* 1992). These authors, recommend the use of perphenazine enanthate to produce reliable sedation of impala, under circumstances where the animals are not exposed to handling.

This study confirms previous reports of changes in haematological and serum biochemical values with capture and housing of wild animals whereas indicate an improvement of the homeostasis of the wild caught otters while in captivity under proper care. Based on the results obtained in this research, perphenazine does not seem to significantly alter the haematological and biochemical parameters involved on the stress response. This does not mean that the use of LANs is not valuable in controlling or decreasing the stress suffered by captured wild animals. Contrary, there are many other factors indicative of stress that were not included in this paper such as body weight increase, food ingestion, daily cortisol level on faeces, behaviour changes and so on, that are worthy to study. Further research concerning these aspects should be design in future.

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Table 1. Values of 17 haematological and serum chemistry parameters performed on three occasions (A,B and C) for the 28 treated and untreated wild caught Eurasian otters.

A: 2-5 days post capture; B: 5-10 days post capture; C: 20-30 days post capture.

Parameters	Number	A		B		C	
		Mean	SD	Mean	SD	Mean	SD
White blood cells (x 10^9/litre)	25	12.31	7.11	10.60	4.40	7.06	3.28
Red blood cells (10^9/litre)	25	6.01	1.09	5.80	0.99	6.52	0.58
Hemoglobin (g/dl)	25	14.7	2.7	13.9	2.4	15.4	1.6
Segmented neutrophils (10^9/litre)	25	8.97	5.54	7.34	3.62	4.5	2.67
Band neutrophils (10^9/litre)	25	0.29	0.48	0.27	0.35	<0.1	<0.1
Lymphocytes (10^9/litre)	25	1.85	1.18	1.78	0.72	0.1	0.25
Monocytes (10^9/litre)	25	0.78	1.14	0.7	0.51	0.44	0.47
Eosinophils (10^9/litre)	25	0.44	0.53	0.45	0.3	0.53	0.33
Basophils (10^9/litre)	25	1.04	2.9	2.16	0.11	1.2	2.76
CK (iu/litre)	27	10056.3	17947.5	911.4	641.2	723.4	435
AP (iu/litre)	26	80.5	45	62.7	30.8	56.3	33.9
ALT (iu/litre)	28	484	390	168.3	132.7	86.2	26.7
AST (iu/litre)	27	764.5	972.9	215.8	106.3	172.2	63.7
LDH (iu/litre)	26	3523.3	2173	1982.7	1039.6	1889.3	1007.2
Albumin (g/litre)	23	27.0	3.1	28.1	3.8	30.7	4.3
Blood urea (mmol/litre)	28	16.8	10.7	13.8	4.0	12.0	3.8
Cortisol (mmol/litre)	21	361.6	1139.8	41.4	30.4	35.9	27.6

Figure 1. Evolution (mean ± SEM) of red blood cell count (RBC) in 28 wild caught Eurasian otters during captivity perio

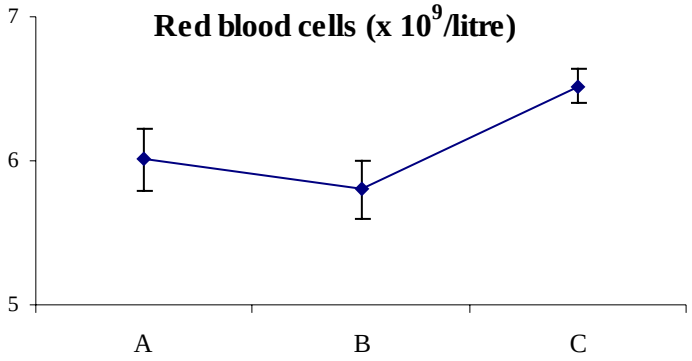


Figure 2. Evolution (mean ± SEM) of haemoglobin (Hb) in 28 wild caught Eurasian otters during captivity period

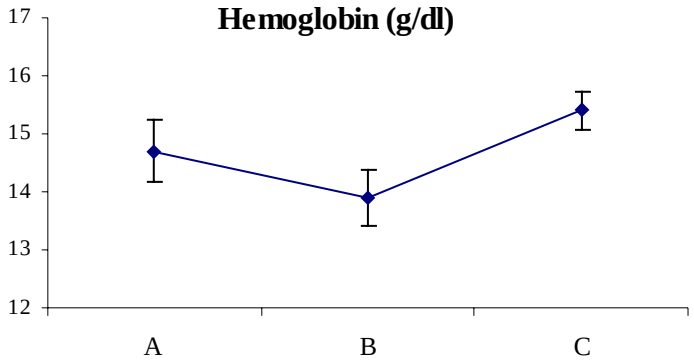


Figure 3. Evolution (mean ± SEM) of white blood cell count (WBC) in 28 wild caught Eurasian otters during captivity period

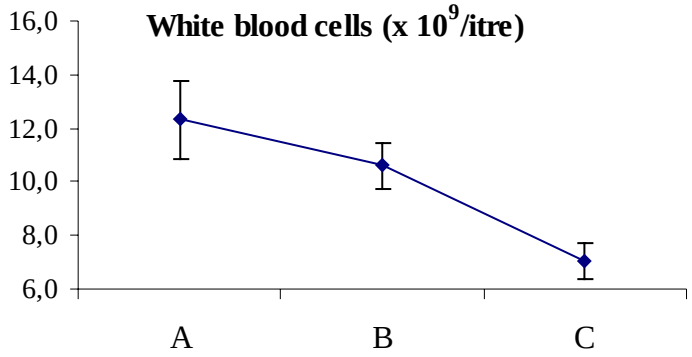


Figure 4. Evolution (mean ± SEM) of segmented neutrophils in 28 Eurasian wild caught otters during captivity period

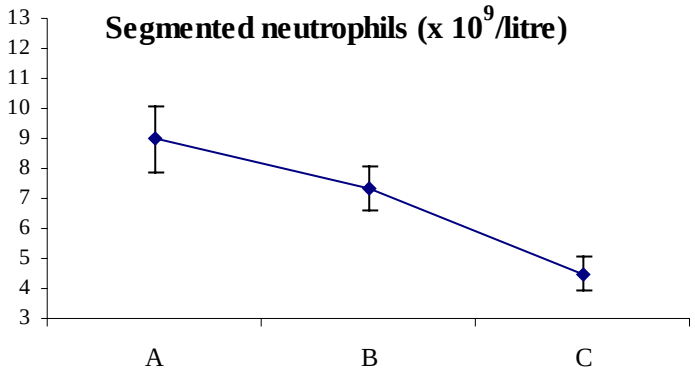


Figure 5. Evolution (mean ± SEM) of blood urea in 28 wild caught Eurasian otters during captivity period

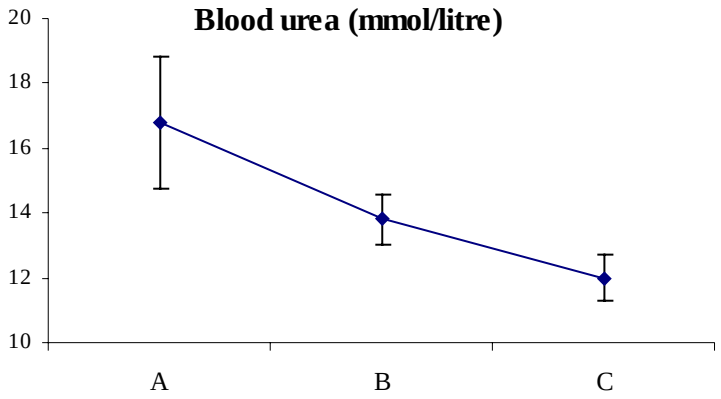


Figure 6. Evolution (mean ± SEM) of alanine aminotransferase (ALT) in 28 wild caught Eurasian otters during captivity period

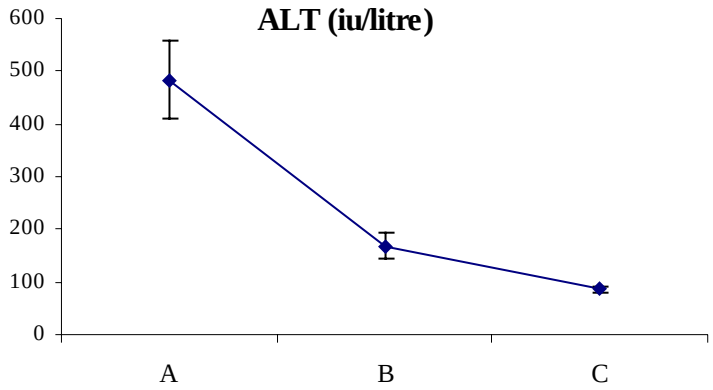


Figure 7. Evolution (mean ± SEM) of aspartate aminotransferase (AST) in 28 wild caught Eurasian otters during captivity period

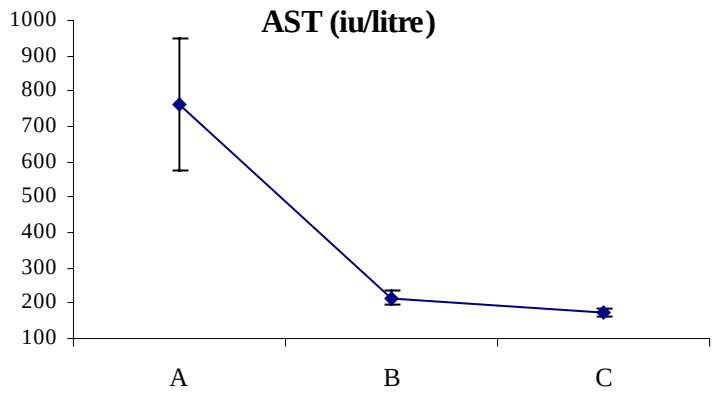


Figure 8. Evolution (mean ± SEM) of creatine kinase (CK) in 28 wild caught Eurasian otters during captivity period

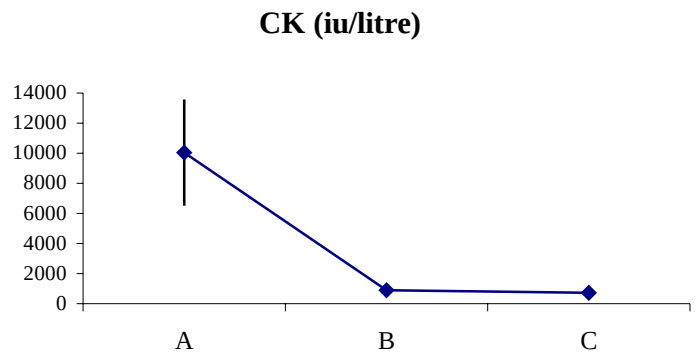


Figure 9. Evolution (mean ± SEM) of lactate dehydrogenase (LDH) in 28 wild caught Eurasian otters during captivity period

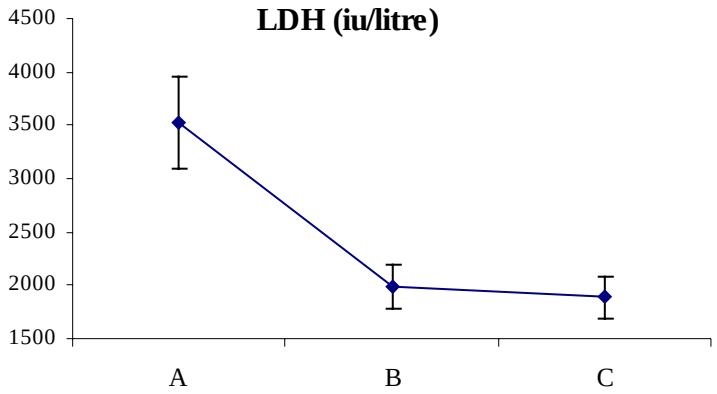


Figure 10. Evolution (mean ± SEM) of alkaline phosphatase (AP) in 28 wild caught Eurasian otters during captivity period

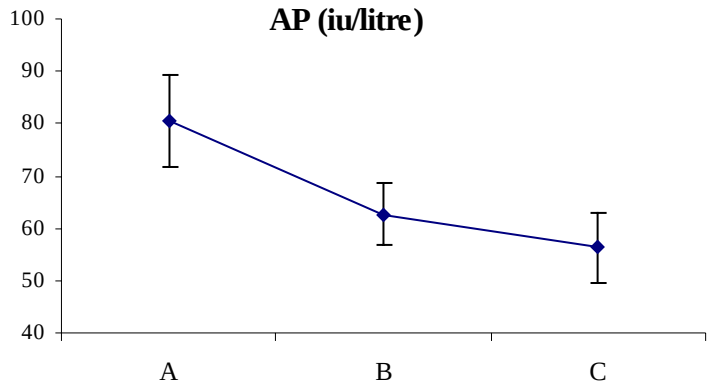
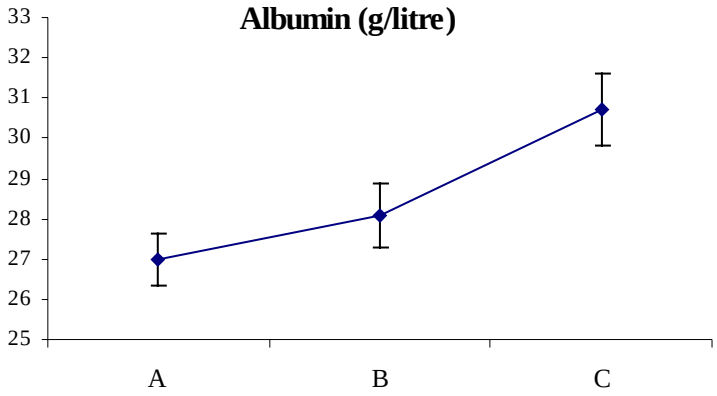


Figure 11. Evolution (mean ± SEM) of albumin in 28 wild caught Eurasian otters during captivity period



El capitulo 6 esta basado en

Perphenazine enanthate usage in wild caught eurasian otters (Lutra lutra) during a reintroduction project: clinical approach and pharmacokynetics. J. Fernandez-Moran, R. Ventura, M. Csarampere, J.Segura, D. Saavedra, X. Manteca-Vilanova. (en preparación).

CAPITULO 6

PERPHENAZINE ENANTHATE USAGE IN WILD CAUGHT EURASIAN OTTERS (Lutra lutra) DURING A REINTRODUCTION PROJECT: CLINICAL APPROACH AND PHARMACOKYNETICS

INTRODUCTION

Animal translocations are considered powerful tools for the management of the natural and man made environments which, properly used, can bring great benefits to natural biological systems and to man. Until today several reintroduction programs have been successfully achieved including the American bison (*Bison bison*), European wisent (*Bison bonasus*), golden lion tamarin (*Leontopithecus rosalia*), black footed ferret (*Mustela nigripes*), and Arabian oryx (*Oryx leucoryx*), among others (Kleiman 1996, IUCN 1995, Fernandez-Moran and others 2001). While the main attention from wildlife veterinarians has focused in identifying medical conditions that could endanger the remaining free-living populations or other animals in the ecosystem to which they are reintroduced (Bush et al. 1993), minor consideration has been dedicated to the stress that reintroduced animals suffer during such programs, specially when wild caught animals are involved (Fernandez-Moran et al. 2002). Veterinarians, as an important part of the multi-professional teams involved in most of the translocation projects, must guarantee that this stress which may cause suffering is reduced to a minimum.

Different methods for reducing stress and mortality in wild animals during their capture, confinement, and transport have been previously reported and include using the appropriate equipment, working with trained personnel, following basic principles, and using tranquilizers appropriately (Ebedes et al. 1998).

Tranquilizers that alleviate the anxiety and stress and have prolonged and safe sedative properties are recommended. The use of short and long acting tranquilizers in wild animals is well documented (Ebedes 1993).

Long acting neuroleptics (LAN) or *depot* tranquilizers have been used for several years in veterinary as well as in human medicine (Ayd 1975, Ebedes 1993). These are neuroleptics with a therapeutic effect lasting for at least 7 days (Ebedes 1993). LAN are formulated by combining the active drug with a long-chained fatty acid, and the combination is hydrolyzed slowly in body tissues, releasing the neuroleptic drug into the vascular system over a prolonged period of time (Ebedes 1993). Among the currently available LAN, perphenazine enanthate (PE) (Trilafon enantat®, Sheering-Plough S.A., Madrid 28046, Spain; 100 mg/ml)), is widely used in the management of free and wild caught animals (Ebedes 1993, Huber et al 2001). Perphenazine is a chlorpromazine analogue and one of a number of phenothiazine derivatives that contain a piperazine ring. The compound was first synthesized in 1956 and has received considerable clinical usage in human medicine since then.

The first report of the use of perphenazine in wild animal is from 1956 (ARNZP). Depot tranquilizers were first evaluated for use in wild animals in 1984 (Ebedes 1993) and although today they are used extensively in zoo and wildlife medicine, we have found no data about pharmacokinetics and serum levels in wild animals. Besides, few reports describe the use of these drugs on animals other than hoofstock and particularly in carnivore species (Holz and Barnett 1996; Huber et al. 2001). Because of this, doses of long acting neuroleptic used on those animals are based on behavioral observations rather than on pharmacokinetics studies. In human medicine, plasma levels of LAN has been used during the past decades to optimize pharmacological treatment, but such studies are not available in zoo and wild animals.

The aim of the present study was to analyze variations of perphenazine concentration in plasma after a single standard dose (20-30 mg per animal) of PE in wild caught Eurasian otters during a reintroduction project conducted in Spain.

MATERIAL AND METHODS

Animals and drugs

During an otter reintroduction project carried out in Norteastern Spain during 1995-2000, 13 adult Eurasian otters (6 males and 7 females) (*Lutra lutra*) weighing from 4.3 to 8.5 kg were wild caught and transported to Barcelona Zoo (BZ, Barcelona, Spain) following procedures described elsewhere (Fernandez-Moran et al. 2002). Trapped animals were chemically

immobilized by manual injection of ketamine and medetomidine intramuscularly (Fernández-Morán et al., 2002). At the moment of capture, and once they were chemically immobilized, these otters were injected i.m. the long acting neuroleptic (LAN) PE at a total dose of 20-30 mg (2.4-5.0 mg/kg). Other 24 otters were included in this project but were not injected with the LAN and therefore could be used as a control group (CG).

After shipment to BZ, otters were individually housed indoors in wire- mesh cages (2.44 m long x 1.22 m wide x 1.22 m high) with attached wooden nest boxes (0.91 m long x 0.61 m wide x 0.51 m high) and suspended above the ground. All otters remained at BZ during a period of between 20 and 30 days in which they were clinically evaluated.

Food and water were offered *ad libitum*. The diet consisted of a mixture of fresh dead or thawed trout, chicks, fresh dead eels, and fresh dead crayfish the first 3-5 days and later on only fresh dead trout.

Any time the otters were immobilized, blood was collected for hematological and biochemical studies as referred elsewhere (Fernandez-Moran et al. 2001). When possible, a plasma sample was submitted for perphenazine determinations. A total of 30 serum samples from treated otters could be submitted for such studies ranging from 1 day to 21 days post perphenazine enanthate administration (Table 1). Eigth serum samples from non-treated otters were collected after 1, 3, 9, 22 and 51 days of captivity.

Although not systematically, we did conduct behavioural observations in all the otters during this study, in an attempt to determine the stress response and sedation.

Sample preparation procedure

Two mls of 1.1 M acetate buffer pH 5.2 was added to 0.5 ml of serum samples and vortex mixed for 5 seconds. When analysing otter serum samples, they were previously centrifuged at 14000 rpm for 10 minutes. Then, 50 μl of ISTD solution (fluphenazine, 0.1 μg/ml) were added to buffered serum samples. Before solid phase extraction, samples were submitted to an enzymatic hydrolysis as follows: 30 μl of β-glucuronidase-arylsulphatase from *Helix pomatia* were added to serum samples, vortex mixed during 5 seconds and incubated at 55ºC for 2 h on a water bath. Then, samples were cooled to room temperature and centrifuged at 3500 rpm for 10 minutes.

Solid phase extraction was performed with Bond Elut Certify columns, which were previously conditioned with 2 ml of methanol and 2 ml of deionised water. Hydrolysed serums were applied in a time up to 2 minutes. Columns were washed with 2 ml of deionised water, 1 ml of 1 M acetic acid and 2 ml of methanol, consecutively, and after drying for 2 minutes, two elutions of 2 ml were carried out with a mixture of chloroform and 2-propanol (80:20 v/v) containing 2% ammonia. The organic extracts were evaporated to dryness under a nitrogen stream in a water bath at 40ºC, kept in a desiccator containing di-phosphorous pentoxide and potassium hydroxide pellets and maintained under vacuum for at least 1 hour.

Dried extracts were dissolved in 30 μl of MSTFA, vortex mixed during 10 seconds and kept at 80ºC for 30 minutes. After cooling to room temperature for 5 minutes, the derivatised extracts were transferred with Pasteur pipettes into injection vials.

Instrumental analysis

The instrumental analysis was performed in a 6890 gas chromatograph coupled to a 5973 mass-selective detector (Hewlett-Packard, Palo Alto, CA, USA) using selected-ion monitoring acquisition mode (SIM), monitoring characteristic ions m/z 246, 372 and 475 for perphenazine derivative and m/z 280 for fluphenazine derivative. The instrument was equipped with a cross-linked 5% phenilmethyl siloxane fused-silica capillary column (12.5 m x 0.2 mm i.d., 0.33 μm film thickness) from Agilent Technologies (USA). Injections were made in splitless mode (2 min delay) using helium as the carrier gas (measured at 180ºC). Injector and transfer line temperatures were set at 280ºC. Initial oven temperature was set at 180ºC, increased at 20ºC/min to 290ºC and maintained for 8 minutes. Total run time was 13.50 minutes. Sample injection volume was 2 μl. Insert liners packed with silanised glasswool were used. For quantitation of otter serum samples, ions corresponding to m/z 246 and 280 for perphenazine and fluphenazine derivatives, respectively, were used.

Calibration curves from 1 to 40 ng/ml were used to quantify perphenazine in serum samples. Each day of analysis control samples prepared by spiking perphenazine in drug-free matrix were analysed in parallel with the otter samples to verify the quantitation procedure.

RESULTS AND DISCUSSION

In table 1 we can find data regarding the serum samples used in this study while Figure 2 illustrates the concentration of perphenazine (ng/ml) in the 30 samples studied. Initial concentration levels ranged from 3.3 to 18.6 ng/ ml within the 4 days post administration. These levels decreased to 3.9 to 4.2 ng/ml in day 7 and afterwards went close to 0. However, perpheanzine could be detected in some animals at days 9, 10, 12, 14, and 17 (1.0, 2.0, 1.1, 0.8, and 0.8 ng/ml respectively) although at very low levels.

Perphenazine was first synthesized in 1956, and three years later it usage in zoo animals was reported in gaur (*Bos frontalis*), yac (*Bos grunniens*), American bison (*Bison bison*), fallow deer (*Dama dama*), and Pampas cat (*Felis pajeros*) (ARNZP 1959). The introduction of depot neuroleptic preparations in the late 1960s opened up new fields in long term treatment of chronic psychotic disorders in psychiatry and have been well documented by Ayd (1975) and Larsen and Hansen (1989). These drugs were first evaluated in wild animals in 1984 by Ebedes (1993).

Most of information of using LAN focuses in African ungulates and dosages in hoofstock range from 0.5-2 mg/kg but there appears to be an inverse relationship between dosage of LAN and the average size of the species with larger species requiring lower dosages per unit weight (Ebedes 1993; Blumer 1991). Crindle et al (1989) used 0.5 mg/kg in two horses and noted an effect for 30 days. In rats, it was demonstrated that 3.4 mg/kg every two weeks during 12 months caused 20% loss of nerve cells in the basal ganglia but when the treatment was only for 2 or 3 months no adverse effects were found (Pakkenberg et al. 1976).

Few authors mention its use in carnivores. Winterer and Wiesner (1998) stated an optimal dosage range for perphenazine enanthate in felids of 0.4- 0.6 mg/kg while Huber et al (2001) found that 3.0 mg/kg produced tranquilization in cheetahs (*Acinonyx jubatus*) with no adverse effects. In humans, where most studies have been carried out, the recommended dosage is 100 once every 14 days (1.2-1.6 mg/kg for a 60-70 kg person).

The standard dosage for otters used in this study (see table 1; 20-30 mgs per animal; 2.4-5.0 mg/kg) was obtained based on previous experiences of authors.

In humans, plasma level monitoring of drugs have been used during the past decades to optimize pharmacological treatment. It is considered to be the best way to verify drug

compliance, specially for neuroleptics. For perphenazine, therapeutic intervals have been established for treatment of acute psychotic episodes although therapeutic intervals for neuroleptic long-term maintenance treatment have not yet been solidly established. Most studies suggest that a stable dose over a very long time is associated with good symptom control and minimal long-term side effects. There are few studies that have looked into the stability of plasma levels of patients on neuroleptic drugs over long time periods (Tuninger and Levander 1996). Besides, serum monitoring is of significant use in finding the optimal dose level and length of interval resulting in a low incidence of side effects and a sufficient therapeutic response as overdosing enhances the risk of developing secondary complications (Larsen and Hansen 1989). In humans, following a single i.m. injection of 100 mg of perphenazine enanthate, perphenazine concentrations averaging 0.001 mg/L are detectable in blood for the 14 days post-drug period. For perphenazine decanoate, the optimal serum level range was establised (2-6 nmol/L) and the peak level was found to occur nearly 7 days after the injection (Larsen and Hansen 1989).

Sedation, epileptogenecity, and extrapyramidal reactions may be numbered among the most important central nervous system effects of neuroleptics. Manifestation of an overdose of perphenazine primarily involves the extrapyramidal system. Moreover, in humans, as stressed by Ayd (1975) there is an individual and sexual predisposition in the development of extrapyramidal symptoms with parkinsonism and akathisia occurring more frecuently in women than in men; acute dyskinesia occurred almost twice as often in men as in women (Ayd 1975). Extrapyramidal effects were not observed in this study.

There is no specific antidote for overdosage with perphenazine and treatment is symptomatic and supportive. Standard measures such as oxygen, intravenous fluids and corticosteroids should be used to manage circulatory shock or metabolic acidosis; body temperature should be regulated, and cardiac function monitored. Convulsions should be treated with anticonvulsants, and Parkinson-like symptoms should be treated with benztropine mesylate or diphenhydramine. Because of these potential complications LAN should be used with caution. Although otters treated in this study were not pregnant (Fernandez-Moran et al 2002) perphenazine has been proved to be teratogenic in pregnant mice and rats, but not in rabbits. As there are no studies in zoo and wild animals, its use in pregnant animals should be limited.

Zoo and wild animals studies about the use of LAN focussed only in behavioral studies or side effects (Huber et al. 2001; Ebedes and Raath 19998; Holz and Barnett 1996) and unfortunately we did not find any pharmacokinetic research to compare our results. Holz et al. 1996 noted that in wallabies (*Macropus rufogriseus*), tranquilization after a single injection of PE declined after 10 days. Huber et al. 2001 evaluated the long-term sedation in cheetah treated with PE, by a behavioral observation protocol and observed a sedative effect from day 1 until day 6 with the maximum effect on day 2.

All otters treated with PE in this study showed apparent sedation signs. They were less aggressive when the wooden box was opened for inspection purposes, and seemed to react less to external stimuli. They were calm and also unresponsive to human presence. These animals did not kill live chicks, whereas control animals did. However they ate fresh dead fish and chicks normally. Although there was a considerable variation in individual behavior, most treated otters appeared much calmer during the 5-7 days after administration than those that had not. In this way, our behavioral observations would correlate with the pharmacokinetics determinations obtained . One animal that had rejected food for 4 days started to eat a few hours after being treated with 20 mg PE i.m. Besides, we did not see any overdose or extrapyramidal symptoms.

Based on these trials, a dose of 2.4-5.0 mg/kg perphenazine enanthate can be recommended for tranquilization of wild Eurasian otters subjected to confinement during translocation programs. No detrimental side effects attributable to this drug was observed during the course of this study. According to this paharmacokinetic study, after a single i.m. administration of perphenazine enanthate in the otter, levels higher than 3 ng/ml can be expected until day 7 post administration.

As far we know this is the first serial quantification of serum perphenazine in wild animals following the application of an standard dosis. Future research combining behavioral observations with pharmacokynetics of LAN are encouraged as these drugs are valuable when managing wild animals.

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Table 1. Information on serum samples used in this study.

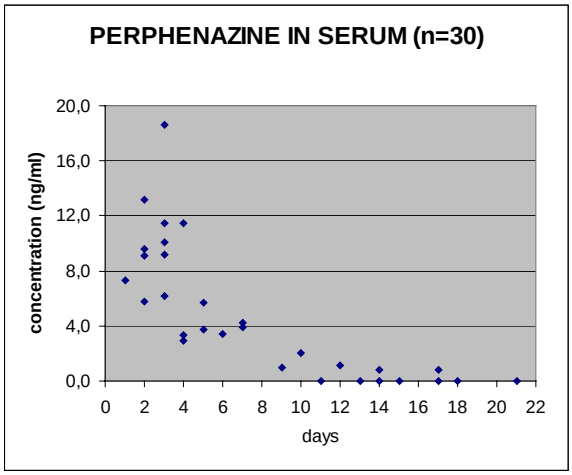
Otter	Sex (M/F)	Time[a]	Weight[b]	Dose[c]
1	M	1,11	8.5	2.4
2	F	2,4	4.3	4.7
3	F	2,7,10	6	5.0
4	M	2,5,7,12,15	4.7	4.3
5	F	2,5,14	5.1	3.9
6	F	3	5.5	3.6
7	F	3,6,17	5.9	3.4
8	F	3,4	5	4
9	M	3,13	8	3.8
10	F	4,9,14	6	3.3
11	M	14	7.8	3.8
12	M	17,21	6.1	4.9
13	M	18	7.8	3.8

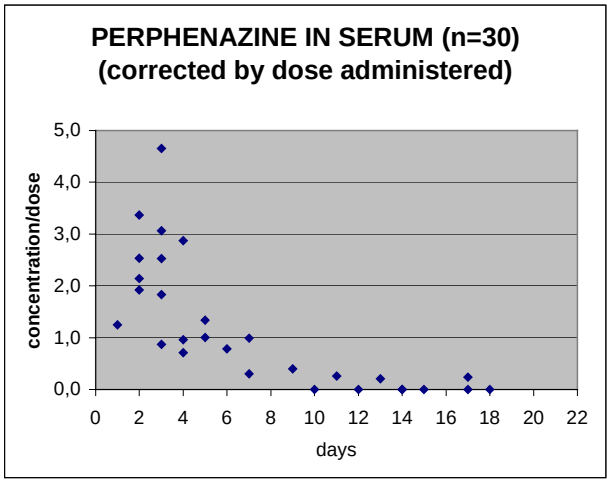
[a] days post- perphenazine injection

[b] Kg

[c] total dose in mg/kg

Table 2. Concentration of perphenazine (ng/ml) in 30 serum samples obtained from wild caught otters in days 1-21 after perphenazine enanthate (PE) administration.





DISCUSION

Intervalos hematológicos y bioquímicos en la nutria eurasiática

La mayoría de los valores hematológicos obtenidos en este estudio son similares a los publicados previamente por Lewis et al. (1998) para la nutria eurasiática en Gran Bretaña. Sin embargo, hemos encontrado algunas diferencias. Nuestros valores de leucocitos (WBC) y neutrófilos fueron superiores, mientras que los de eosinófilos y linfocitos fueron inferiores. Estas divergencias con el trabajo de Lewis et al. (1998) bien podrían deberse al modo de obtención de la muestra; en nuestro caso los animales eran totalmente salvajes, por lo que para manipularlos recibían una dosis anestésica mediante cerbatana. En el estudio de Lewis, los animales procedían de un centro de recuperación en el que habían permanecido al menos varios meses, por lo que los animales estaban más acostumbrados a la manipulación. Como bien reflejan Meyer et al. (1992), el estrés puede inducir a cambios en el hemograma tales como leucocitosis y neutrofilia. Además, en otras especies de carnívoros se han encontrado diferencias en el leucograma (neutrofilia, leucocitosis, monocitosis, linfopenia, eosinopenia, etc.) dependiendo de si los animales habían sido recién capturados del medio natural o si, en cambio, habían permanecido previamente en cautividad (Fuller et al. 1985; Beltran et al. 1991).

Nuestros resultados en el número de plaquetas también difieren de los observados por Lewis et al.(1998). Según este autor, los individuos juveniles mostraban contajes muy superiores. En su trabajo, si consideramos únicamente a los individuos mayores de un año, los valores obtenidos son similares a los nuestros.

Tanto la aspartato aminotransferasa (AST) como la creatin quinasa (CK) fueron más elevadas en nuestro estudio. La causa de estas discrepancias puede estar en el hecho de que nuestros animales habían sido capturados del medio salvaje mediante trampas unos 20 días antes. El daño muscular que se produce cuando un animal es capturado puede dar lugar a un incremento en las actividades de la AST y la CK (Seal et al. 1975), aunque en nuestro caso esto resulta poco probable ya que la vida media en plasma de estas enzimas es corta (Kramer 1989). Por lo tanto, nuestros valores superiores seguramente se deben a contaminación parcial de las muestras con fluidos intracelulares de músculo esquelético (MacWilliams & Thomas 1992) producida al intentar pinchar repetidas veces con una misma aguja. Por último, en el trabajo de Lewis et al. (1998) no se mencionan los reactivos empleados para llevar a cabo la determinación

de las actividades de estas enzimas y es bien sabido que esto puede inducir diferencias en los resultados.

Finalmente, tanto el colesterol como la urea en sangre (BUN) difieren en ambos trabajos, pero esto puede estar justificado por las diferencias en la dieta entre los dos grupos estudiados (Williams & Pulley 1983; Ruiz-Olmo & Palazon 1997).

Nuestros resultados confirman que la nutria euroasiática presenta menos hematies, pero con mayor volúmen corpuscular medio (VCM) y hemoglobina corpuscular media (HCM) que la nutria americana (Lewis et al. 1998). Esto es difícil de explicar ya que ambas especies presentan hábitos de alimentación y buceo similares.

No encontramos diferencias significativas en ninguno de los parámetros estudiados (salvo en la albúmina y las plaquetas) cuando comparamos machos y con hembras, lo cual coincide con los estudios previos en nutria americana y eurasiática (Lewis et al. 1998; Todcilowski et al. 2000).

Anestesia de la nutria eurásiatica mediante la combinación de medetomidina y ketamina y su antagonización con atipamezol

La ketamina ha sido empleada sola o combinada con alfa-2-agonistas o benzodiazepinas, tales como xilacina, diazepam, midazolan y medetomidina, en una gran variedad de carnívoros (Kreeger et al. 1996; Ramsden et al. 1976). En las nutrias americanas y euroasiáticas, varios autores recomiendan dosis de ketamina sin combinar de 6-30 mg/kg (Jenkins & Gorman 1981; Kuiken et al. 19888; Reuther & Brandes 1984; Serfass et al. 1993). En la nutria americana dosis de ketamina de 10 mg/kg resultan en anestesias con un grado de calidad variable, miorelajación pobre, hipertermia, movimientos y complicaciones cardiopulmonares, por lo que la ketamina sola no se recomienda en esta especia (Spelman et al. 1993). Sin embargo, cuando se combina con medetomidina mejora la relajación muscular aumentando la profundidad anestésica a la vez que se obtiene reversibilidad (Spelman et al. 1993). Las combinaciones de ketamina y xilacina fueron empledas por primera vez en animales salvajes por Jalanka y Roeken (1990) quienes describe su uso seguro, eficaz y práctico en una gran variedad de mamíferos (Jalanka y Roeken 1990; Jalanka 1989). En la nutria europea, las guías de manejo de la EEP (Programa Europeo de Cría) (Vogt 1994) recomendaban entre otras, la combinación de medetomidina (150 μg/kg) con

ketamina (5-10 mg/kg) o medetomidina (100 μg/kg) con ketamina (5 mg/kg) y midazolan (0.2 mg/kg). En contraste, en la nutria americana, Spelman (1999) empleó con éxito dósis tan bajas como 25 μg/kg de medetomidina con 2.5 mg/kg de ketamina obteniendo una anestesia de corta duración estable y adecuada. Cuando la misma autora empleo dosis ligeramente superiores de ketamina (10 mg/kg) con xilacina (1-2 mg/kg), observó una importante depresión respiratoria (Spelman 1999). Lewis (1991) describe la anestesia adecuada de 10 nutrias asiáticas mediante la combiánción de medetomidina (100-120 μg/kg) y ketamina (4-5 mg/kg).

La dosis claramente inferior de medetomidina (50 μg/kg) empleado en nuestro estudio se basó inicialmente en los estudios previos realizados por Spelman et al. en 1993 con la nutria americana, así como en experiencias no publicadas de los autores. Cuando probamos dosis inferiores no conseguimos un plano anestesico adecuado para el manejo de estos animales y por el contrario, a dosis superiores detectamos depresión respiratoria severa (frecuencia respiratoria inferior a 10 respiraciones por minuto y saturación de oxígeno relativa en sangre arterial ($SpO_{2)}$ inferior al 80%). En combinación con la medetomidina, los efectos anestésicos de la ketamina son potenciados, lo cual permite disminuir su dosis. Esto resulta en una mejor calidad anestésica (mejor miorelajación) a la vez que facilita la antagonización de la anestesia mediante el uso de alfa-2-antagonistas como la tolazolina o el atipamezol (Jalanka 1989). En nuestro estudio, 5 mg/kg de ketamina resultaron eficazes para alcanzar este propósito.

Según algunos autores, el atipamezol puede causar excitabilidad en algunos carnívoros tratados con medetomidina y medetomidina-ketamina (Jalanka & Roeken 1990), quizás como consecuencia del efecto residual de la ketamina. En nuestro estudio, la mayoría de los animales se recuperaron de manera suave y gradual.

Una de las principales complicaciones anestésicas en la nutria asociada al uso de la ketamina es la hipertermia (Reuther & Brandes 1984), pero en nuestro estudio no la apreciamos debido al empleo de dosis bajas y a la combinacion con la medetomidina.

Las nutrias americanas son muy sensibles al efecto depresor de la ketamina sobre su sistema respiratorio (Spelman et al. 1993). En perros, la medetomidina disminuye la frecuencia respiratoria y puede alterar los patrones respiratorios, pero al combinarla con ketamina se

atenuan estos efectos (Kreeger et al. 1996). En nuestro estudio, no apreciamos depresión respiratoria y las nutrias mostraron en todo momento respiraciones profundas y regulares. En nutrias, los valores de SpO_2 por debajo del 90% se consideran una complicación anestésica, indicando depresión cardipulmonar (Spelman et al. 1997). En nuestro caso, la SpO_2 media fue superior (93%) aunque en un caso alcanzó el 73%. De todas maneras, siempre que se empleen anestésicos inyectables en nutrias, se aconseja la suplementación con oxígeno por medio de mascarilla o tubo nasal (Spelman et al. 1997) y tener a disposición un tubo endotraqueal adecuado por si hubiera depresión respiratoria severa. En condiciones de campo, si surgieran complicaciones, se aconseja aplicar rápidamente el antagonista y mantener al animal en observación en un lugar tranquilo, oscuro y controlado durante unos minutos (hasta su total recuperación).

No hemos encontrado información sobre la frecuencia cardíaca en la nutria eurasiática, pero Spelman (1993) se refiere a bradicardia en la nutria americana cuando el ritmo cardíaco cae por debajo de 100 pulsaciones por minuto. Según este dato, la bradicardia fue una constante en nuestras nutria anestesiadas, ya que en un 39% de la inmobilizaciones observamos valores inferiores. A pesar del efecto estimulante de la ketamina sobre el sitema cardiovascular, al emplearse la combinación de ketamina-medetomidina puede ocurrir un efecto final depresivo (Spelman et al. 1994). El uso sitematico de anticolinergicos (atropina) junto con la medetomidina para contrarestar la bradicardia es un hecho polemico (Kreeger et al. 1996; Spelman 1999) ya que puede inducir hipertensión, la cual puede verse agravada si además de medetomidina empleamos ketamina. En nuestro estudio los animales respondieron de manera positiva a la atropina (0.02 mg/kg), incrementándose el ritmo cardíaco tras su administración. No obstante, al no monitorizar la presión arterial no podemos saber exactamente la importancia o efectos negativos de su uso.

Captura

Los métodos de captura empleados en este estudio son similares a los empleados en proyectos similares en los EEUU (Serfass et al. 1996) con un índice de captura (numero de nutrias capturadas dividido por el numero de capturas potenciales) similar (0.57 vs 0.60), entendiéndose como capturas potenciales la suma de las capturas y los escapes de las trampas. Sin embargo en nuestro caso se necesitaron 159 trampas por animal capturado en contraste con las 60 que precisó Serfass (1996). En el programa de Carolina del Norte (Spelman 1998) el

número de trampas empleadas fue incluso inferior (26 por nutria). Se ha sugerido que estas variaciones pueden deberse a la diferencia de comportamiento entre ambas especies de nutrias (*Lutra lutra vs Lontra canadensis*) así como a factores relacionados con las zonas de captura. En nuestro estudio también se observaron diferencias entre las diferentes áreas de captura.

Durante este proyecto, las hembras gestantes o en periodo de lactación y las crías eran liberadas y desestimadas para su traslado al Zoológico. Sin embargo uno de los animales que murió fue un individuo subadulto que fue incluido debido a su tamaño y a su aspecto general de animal casi adulto. Este animal nunca se adaptó a las condiciones de cautividad y rechazó la comida. Por este motivo se recomienda evitar translocar individuos que no alcancen el tamaño de adulto.

Los animales capturados en este estudio sufrieron diversas lesiones y aunque en la mayoría de los animales sólo se observaron abrasiones, inflamaciones en extremidades y pérdida parcial de alguna uña, el 21% (9 animales) se vio afectado por algún tipo de luxación en las falanges. Este tipo de lesiones ha sido descrito previamente y es común con el empleo de trampas cepo con bordes acolchados (*Softcatch*), las cuales son consideradas adecuadas para la captura “humana” de nutrias (Serfass 1993). No pudimos comparar este dato con otros proyectos, ya que la metodología empleada en cada caso no está estandarizada. En la mayoría de los casos el animal se autoinflinge estas lesiones en sus intentos por escapar de la trampa. Comparado con otros estudios, hemos observado menos incidencia de lesiones dentales (Serfass 1993), únicamente apreciándose éstas en un 19% (8 animales) lo cual puede deberse a las diferencias de comportamiento de las diferentes nutrias capturadas. Así como en otros estudios las nutrias mordían los cepos (Serfass 1993), en nuestro caso se centraban en huir, escarbar y destruir la vegetación circundante.

Uno de los aspectos más positivos instaurados a lo largo del proyecto fue el empleo de la cerbatana para inmovilizar químicamente a las nutrias capturadas en los cepos. Inicialmente se manipulaban a mano y el anestésico se aplicaba manualmente, lo cual constituía una situación altamente estresante y traumática para los animales. Por otra parte, el esfuerzo y los movimientos realizados por las nutrias empeoraban o agravaban las lesiones producidas por las trampas, como luxaciones y fracturas. Posteriormente se cambio de táctica. Una vez que los operarios apreciaban que había una nutria en el cepo, se preparaba un dardo con la droga en función del peso estimado y éste era lanzado mediante cerbatana desde una distancia de 1-2 metros. A partir

de los 3 minutos la mayoría de los animales podían ser manipulados con seguridad para ellos o los operarios. Aunque se ha propuesto el empleo de redes, mantas y jaulas y cajas de contención (Serfass et al. 1993; Spelman 1998; Williams y Sniff 1983), creemos que el empleo de la cerbatana para aplicar tranquilizantes sin necesidad de manipular a los animales es el mejor método para retirar a los animales de los cepos o trampas.

Alojamiento y cuidados en cautividad

En general las nutrias de este proyecto se adaptaron bien a las nuevas condiciones y a la presencia humana. La alimentación durante los primeros días consistente en pollitos y pescado vivo (trucha y anguila) fue determinante para que algunos animales que inicialmente rechazaban el alimento comenzaran a comer. Cada vez que los animales debían ser manipulados eran previamente anestesiados mediante el protocolo anestésico desarrollado durante este proyecto lo cual redujo ostensiblemente el estrés de los animales.

Cirugía

Todos los animales operados para la inserción de un radioemisor intraperitoneal (36 individuos) comieron normalmente el mismo día en que fue realizada la intervención quirúrgica, lo cual refleja la escasa incidencia que este hecho tuvo sobre el comportamiento de los animales. La herida quirúrgica nunca se infectó o dañó durante el postoperatorio y ningún animal se la rascó, lamió, o mordió para retirarse los puntos.

Los radioemisores empleados en este estudio (30-40 gramos) son bastante más pequeños que los empleados en los proyectos iniciales en la nutria (110-120 gramos) (Arnemo 1991; Hoover 1984; Reid et al. 1986). Existe cierta controversia en cuanto al tipo de aboradje quirúrgico para esta intervención, así como la duración del periodo en cautividad postquirurgico. En nuestro caso, recomendamos la linea media ventral, aunque otros autores empleen el lateral (Melquist & Hornocker 1979; Serfass et al. 1993). En cuanto al tiempo de recuperación, estimamos que los diez días empleados en este estudio son los adecuados para corroborar una cicatrización completa de la herida y descartar complicaciones postquirurgicas. En la nutria americana, se ha constatado que algunos individuos frotan violentamente el abdomen con el suelo después de la cirugía lo cual puede resultar en una mala cicatrización o incluso en la

abertura de la incisión (Melquist & Hornocker 1979; Williams & Sniff 1983; Serfass et al. 1993). Este hecho nunca fue observado en nuestras nutrias.

Reproducción y mortalidad post-libración

En la nutria americana y según los estudios de Reid et al. (1986), la aplicación de los radioemisores intraperitoneales no tuvo ningún efecto negativo en la reproducción. En nuestro caso, al menos tres hembras operadas y liberadas se han reproducido con éxito. Por otra parte la radiolocalización ha permitido a los biólogos conocer en cada momento los movimientos y distribución de los animales liberados, y así determinar, en su caso, las causas de muerte. Los accidentes de tráfico son responsables del 83% de las muertes en nutria del sudeste de Inglaterra y de un 70% en el sur de Irlanda (Simpson 1997). En nuestro caso, de los nueve animales muertos durante el primer año de su reintroducción, el 56% murió víctima de los coches, un 11% por artes de pesca ilegales, un 11% por un sifón en un canal y un 11% por envenenamiento, lo cual demuestra que también en Cataluña, en el área del estudio, las carreteras son la principal causa de muerte de este mustélido. Las diferencias observadas con los resultados publicados por Simpson (1997) probablemente se deban a que en sus estudios, unicamente se reflejan los animales encontrados por azar y referidos a los centros de rescate de fauna. En nuestro caso, algunos de los animales muertos lejos de carreteras o nucleos de poblacion solo pudieron ser localizados gracias a los radioemisores que llevaban implantados. De no haber sido asi, el porcentaje de animales muertos por accidentes de trafico en las nurtrias liberadas en Cataluña hubiera sido similar al dado por Simpson.

El estrés en la nutria: efectos del tiempo en cautividad y la perfenazina

Como ha sido citado en la introducción, en los animales salvajes recién capturados se han descrito dos tipos de estrés: (1) el estrés primario de corta duración, que se inflinge a un animal salvaje que es perseguido, capturado o manipulado físicamente y (2) el estrés secundario o de larga duración, que es aquel que inducimos a los animales durante el transporte, confinamiento y la adaptación (temporal o permanente) a la cautividad (Nielsen 1999). En nuestras nutrias puede resultar dificil separar o diferenciar claramente ambos tipos de respuesta ya que es previsible que durante el tiempo que permanecieron en cautividad y mientras se las sometía a revisiones veterinarias se solaparan ambas.

Se han citado una serie de parámetros sanguíneos como “indicadores del estrés”: leucocitosis, neutrofilia, incrementos en AST, ALT, LDH y CK, así como descenso en la albúmina.

En nuestras nutrias, las diferencias estadísticas en relación al tiempo en cautividad observadas en los hematíes y la hemoglobina son difíciles de explicar. Estos dos parámetros comenzaron elevados en la primera muestra, disminuyeron en la segunda y finalmente se incrementaron. En animales domésticos, se citan como causa del descenso de estos parámetros, la anemia, final de gestación, tranquilización, anestesia, y hemólisis (Bush 1991). Es posible que el proceso de captura, transporte y aclimatización a las instalaciones del Zoo de Barcelona produjera una ligera anemia de la que los animales se recuperaron rápidamente. Sin embargo, los valores obtenidos en las tres muestras se encuentran dentro de los intervalos descritos por Lewis et al. (1998) para la nutria eurasiática.

Además, observamos contajes elevados de leucocitos y neutrófilos que disminuyeron gradualmente a lo largo del estudio. El efecto del estrés sobre los leucocitos varía según la especie y depende de la distribución normal relativa de leucocitos. Por otra parte en varias especies de carnívoros y ungulados, la leucocitosis y la neutrofilia, han sido atribuidas al estrés de captura (Kreeger et al. 1990; Rietker et al. 1994; Weaber & Johnson 1995), lo cual sugeriría que en nuestras nutrias la respuesta al estrés disminuía conforme aumentaba el tiempo en cautividad. Además, nuestras nutrias podrían estar afectadas por lesiones o heridas producidas durante la captura o el traslado que habrían mejorado con el tiempo, resultando por lo tanto en un descenso en el número de leucocitos. El tratamiento con antibióticos de las nutrias que presentaban lesiones infectadas o proclives a tenerlas, así como de todas las nutrias en el momento de la cirugía (profilaxia postoperatoria), también puede haber influido en este hecho. Los valores finales (muestra C) también están en concordancia con los de Lewis et al. (1998).

El estudio de la creatin quinasa (CK) sérica en animales salvajes es especialmente útil, ya que su incremento está relacionado con degeneración muscular y/o mionecrosis activa o muy reciente. Las nutrias capturadas recientemente en nuestro estudio, presentaban valores muy elevados de esta enzima, lo cual indica que estaban muy estresadas.

El estrés puede inducir, como consecuencia del aumento del catabolismo de las proteinas, hipoalbuminemia. En nuestro caso, la fracción de albumina se incrementó durante el cautiverio

posiblemente como consecuencia de la reducción del estrés, aunque también es posible que la causa estuviera relacionada con los cambios en los hábitos alimentarios, lo cual también explicaría el descenso en la urea (BUN).

Los niveles de cortisol en sangre también han sido extensivamente empleados como un indicador del estrés (Harlow et al. 1987; Morton et al. 1995; Parrot et al. 1994) junto con otros parámetros. En nuestro caso, no observamos diferencias estadísticas entre los niveles en cada una de las tomas, probablemente porque este parámetro estaba consistentemente elevado en cada una de ellas con motivo de la respuesta primaria al estrés relacionada con la inyección del dardo para anestesiar a las nutrias cada vez que eran manipuladas. Ademas, los niveles de cortisol en sangre presentan una gran variabilidad dependiendo de factores no relacionados con el estrés (como la hora del hora del día) (Morton et al. 1995)

Existe cierta controversia sobre el momento de liberar animales tras ser capturados en los proyectos de translocación. La American Association of Mammalogists en sus guías para la captura, manejo y cuidado de mamíferos (ASM 1998) recomienda que los animales translocados sean liberados tan pronto como sea posible después de la captura para minimizar el estrés resultante de las condiciones de cautividad. Siguiendo este patrón, Arnemo (1991) y Hoover et al. (1985) mantuvieron sus nutrias durante un máximo de cinco días tras la implantación del radioemisor. Nosotros seguimos la misma metodología descrita por Serfass et al (1996) quien mantuvo las nutria en observación tras la captura, durante un periodo variable entre 10 y 14 días. Durante este tiempo, sus nutrias fueron evaluadas, implantadas y tratadas de lesiones previas a la captura o producidas durante ésta. Aunque ellos no evaluaron en sus estudios el estrés, estimaron que los cuidados previos a la liberación beneficiarian a los animales a la hora de enfrentarse con un nuevo medio. Los datos presentados en este estudio muestran que un periodo de observación, descanso, recuperación y cuidado antes de la liberación puede ser beneficioso para la reintroducción adecuada de los animales en un nuevo medio. Nuestros resultados parecen indicar que el estrés causado por la captura y por los procedimientos quirúrgicos disminuye con el tiempo, mientras el animal está en cautividad, siempre y cuando las condiciones de mantenimiento sean las adecuadas. Así, cuando el animal ha sufrido un estrés muy intenso durante la captura o las manipulaciones posteriores –como era el caso de nuestras nutrias- podría ser conveniente retrasar la liberación unos días para permitir su recuperación. En las nutrias del presente estudio**,** los principales parámetros relacionados comúnmente con el estrés variaron

gradualmente hacia valores fisiológicos descritos para esta especie, lo cual indicaría un descenso gradual de éste.

La perfenazina fue sintetizada por primera vez en 1956 y tres años después se cita su uso en animales salvajes: gaur (*Bos frontalis*), yac (*Bos grunniens*), bison americano (*Bison bison*), gamo (*Cervus dama*) y gato de la Pampa (*Felis pajeros*) (Morris & Harris 1960). La introducción de las preparaciones depot (LAN o Long Acting Neurolepticos) en los años 60 abrió nuevos puertas en el campo de psicofarmacología y su uso en psiquiatría se extendió rápidamente como ha sido descrito por Ayd (1975) y Larsen & Hansen (1989). Estas drogas se evaluaron por primera vez en animales en 1984 (Ebedes 1993), cuando fueron testadas en una gran variedad de artiodactilos sudafricanos en capturas masivas. Desde entonces su empleo en animales salvajes y de zoo se ha generalizado durante el manejo de animales, pero sorprendentemente, su empleo en no ungulados permanece sin estudiar y no disponemos más que de un par de citas sobre el empleo de LAN en carnívoros (Huber et al. 2001; Winterer & Wiesner 1998).

En medicina humana, en la que se han llevado a cabo numerosos estudios, se recomienda una dósis estándar de 100 mg cada 14 días (1.2-1.6 mg/kg para una persona de 60-70 kilos). Sin embargo, en veterinaria, las dosis empleadas en cada especie son muy variables y no se basan en estudios científicos, sino en apreciaciones subjetivas de diferentes autores. En ungulados, las dósis fluctuan entre 0.5-2 mg/kg pero parece haber una relación inversa entre la dósis del LAN y el tamaño de las especies, precisando las especies menores dósis sustancialmente superiores a las de mayor tamaño (Ebdes 1993; Blumer 1991). Winterer & Wiesner (1998) recomendaron en felinos dósis de 0.6 mg/kg mientras Huber et al (2001) emplearon satisfactoriamente en guepardos 3.0 mg/kg.

En nuestro estudio, y basándonos en experiencias previas en la nutria, empleamos dósis de 2.4-5.0 mg/kg.

No hemos encontrado ninguna referencia sobre el empleo de neurolépticos de larga duración (LAN) en nutrias, por lo que la dosis empleada en este estudio se basó en las observaciones clínicas no publicadas llevadas a cabo por el autor.

Aunque la perfenazina, a las dósis empleadas en este estudio, no suprimiera las respuestas fisiológicas analizadas frente al estrés, esto no significa que su aplicación no fuera beneficiosa para las nutrias. De hecho, apreciamos una clara tranquilización que permitía acercarse a las nutrias sin que atacaran o se espantaran; toleraban mejor la presencia humana. Esto mismo ha sido descrito en pacientes humanos y en animales salvajes tratados con LAN (Knox et al 1992). Los mismos autores recomiendan el uso de perfenazina enantato para la sedación de impalas que no han de ser manipulados.

Entre los principales efectos sobre el sistema nervioso central de estas drogas se incluyen entre otros la sedación, reacciones epileptiformes y reacciones extrapiramidales. El principal signo de sobredosificación es la aparición de síntomas extrapiramidales: catatonia, Parkinson, anorexia o ingestión compulsiva de alimentos, torsión del cuello, sopor, convulsiones, temblores y salivación. Ninguno de ellos fue apreciado en los animales de este estudio. Esto es importante, ya que no existe un tratamiento específico para la intoxicación con estos agentes (excepto tratamiento sintomático y de mantenimiento), lo cual nos debe alertar sobre el uso indiscriminado de esyos productos.

En medicina humana los tratamientos con LAN han sido monitorizados mediante los niveles séricos, con el fin de optimizar el tratamiento farmacológico (obtener la dósis mínima efectiva) y así se establecieron los intervalos terapeuticos de la perfenazina enantato para el tratamiento durante los episodios psicóticos (Tuninger & Levander 1996). En personas, después de una inyección intramuscular profunda de 100 mg de perfenazina enantato, se detectan concentraciones medias de 0.001 mg/L en sangre durante un periodo de 14 días (Larsen & Hansen 1989).

En animales salvajes, todos los estudios de que disponemos sobre el uso de LAN, valoran su efecto y duración, basándose únicamente en estudios etológicos (Ebedes & Raath 1998; Holz & Barnett 1996; Huber et al 2001). No encontramos ningún estudio farmacocinético con el que comparar nuestros resultados.

Creeemos que este es el primer estudio en animales salvajes en el que se realiza una cuantificación seriada de perfenazina en suero, después de la aplicación de una dósis estándar. Aunque creemos que la valoración y el ajuste de la dósis del Trilafon en animales salvajes no se podría realizar rutinariamente mediante la monitorización de los niveles del fármaco en sangre,

estudios de este tipo son muy útiles para establecer dósis seguras y efectivas para las diferentes especies. Por otra parte, el empleo de los LAN se extiende cada vez más porque permite dosificar un fármaco durante un gran periodo de tiempo mediante la aplicación de una sola dósis. De esta manera se simplifica la administración y se inflinge un menor estrés al animal a tratar. Por este motivo creemos que es fundamental saber cuantos días permanecen estos fármacos en circulación a niveles efectivos en las diferentes especies.

En base a nuestros estudios, hemos demostrado que el enantato de perfenazina (2.4-5.0 mg/kg) puede ser recomendada para la tranquilización de nutrias. Tras una única administración en el momento de captura, pudimos detectar niveles séricos superiores a 3 ng/ml durante un mínimo de 7 días.

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CONCLUSIONES

1- La técnica quirúrgica descrita con abordaje en línea media ventral para la implantación de los radioemisores en nutria se considera segura y práctica para su empleo en nutrias ibéricas, como parece deducirse de los 39 animales intervenidos sin ninguna complicación asociada.

2- Los intervalos de referencia hematológicos y bioquímicos de la nutria ibérica han sido establecidos. Los resultados son similares a los descritos previamente para la nutria euroasiática excepto valores ligeramente superiores para los leucocitos, neutrofilos, aspartato aminotransferasa (AST) y creatin kinasa (CK) e inferiores para los eosinófilos y linfocitos.

3- En contraste con la nutria americana (*Lontra canadensis*), la nutria euroasiática presenta menos eritrocitos asociados a un volumen corpuscular medio y una hemoglobina corpuscular media más elevados.

4- Para todos los parámetros sanguíneos estudiados, excepto en la albúmina y las plaquetas, no hemos observado diferencias significativas entre machos y hembras.

5- La combinación anestésica consistente en la mezcla de ketamina (5 mg/kg) con medetomidina (50 μg/kg) se considera segura y efectiva para su empleo en nutrias salvajes. Se recomienda su uso durante proyectos de translocación de esta especie que requieran la inmovilización química de ejemplares durante su desarrollo.

6- Aunque esta combinación es segura, rápida y reversible mediante atipamezol (250 μg/kg), puede provocar depresión cardíaca y bradicardia.

7- Los principales parámetros hematológicos y bioquímicos relacionados con el estrés (RBC, Hb, leucocitos, neutrofilos, AST, ALT, CK, AP y LDH) disminuyeron significativamente durante el tiempo que las nutrias permanecieron en cautividad, lo que significa que el estrés de los animales disminuyó conforme se adaptaban a sus nuevas condiciones de vida en el zoológico. Por este motivo se recomienda, en programas similares, mantener a los animales

unos días en cautividad previamente a la suelta con el fin de liberarlos en mejores condiciones fisiológicas.

8- El uso del enantato de perfenazina (Trilafon®) a las dósis de 2.4-5.0 mg/kg, no influyó en ninguno de los parámetros hematológicos y bioquímicos estudiados.

9- En la nutria ibérica, el enantato de perfenazina a dosis entre 2.4-5.0 mg/kg produce un efecto de sedación moderada que perdura entre 5 y 7 días. Durante este periodo es posible detectar el fármaco en muestras de plasma (niveles superiores a 3 ng/ml). Por lo tanto puede concluirse que el efecto del enantato de perfenazina tiene una duración aproximada de una semana en la nutria Euroasiática. A las dosis indicadas no se observaron efectos adversos.