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Relación entre los problemas médicos y los problemas de comportamiento en el perro y el gato doméstico

Memoria presentada por **Tomàs Camps Morey**

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FACULTAT DE VETERINÀRIA

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CERTIFICA:

Que la memoria titulada "**Relación entre los problemas médicos y los problemas de comportamiento en el perro y el gato doméstico**", presentada por Tomàs Camps Morey para optar por el grado de Doctor, se ha realizado bajo su dirección y, considerándola acabada, autoriza su presentación para que sea juzgada por la comisión correspondiente.

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"*La verdadera ciencia enseña, sobre todo, a dudar y a ser ignorante*"

Ernest Rutherford (1871 – 1937)

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"Estàs segur que vols ser menescal de gran?

Pensa que si un dia te duen un cocodril amb mal de queixal ho tendràs fotut"

Idò sí pradí, en es final me vaig fer menescal.

ÍNDICE DE CONTENIDOS

RESUMEN

El objetivo de esta tesis es estudiar la relación entre los problemas de comportamiento del perro y el gato doméstico y su estado de salud. Consta de dos grandes partes. La primera estudia el papel de los problemas médicos sobre el comportamiento del animal. En la segunda se analiza la relación inversa.

El comportamiento de cualquier animal depende directamente del funcionamiento de sus órganos y sistemas. Muchos problemas médicos pueden modificar su funcionamiento. Por lo tanto, los problemas médicos pueden alterar el comportamiento del animal.

El dolor puede potenciar la aparición, por diferentes mecanismos, de miedo, agresividad, conductas compulsivas y afectar al sueño. Además, se observó que existen dos formas clínicas diferentes de expresar la agresividad por dolor en el perro. Los perros que no son agresivos e inician un proceso doloroso, suelen ser agresivos cuando van a ser manipulados. Además estos son impulsivos, es decir, no muestran señales previas antes de morder. En el caso de aquellos perros que ya son agresivos cuando se inicia el proceso doloroso, suelen empeorar la agresividad pre-existente.

Muchos de los problemas médicos se expresan únicamente con cambios de comportamiento, incluso, sin alterar las pruebas de laboratorio o de imagen. Este hecho es especialmente importante en algunos problemas neurológicos que cambian el comportamiento. Existen zonas del sistema nervioso central (SNC) que tienen un control sobre el comportamiento pero que, sin embargo, no se reflejan en el examen neurológico. Además, algunas lesiones del SNC pueden ser únicamente funcionales, por lo que no se detectan ni en el examen neurológico, ni en las pruebas de imagen usadas convencionalmente. Finalmente, también existen problemas neurológicos que dan alteraciones en el examen neurológico y problemas de comportamiento pero que, sin embargo, no modifican las pruebas complementarias. La tesis recoge uno de estos casos, un gato con una polioencefalomielopatía espongiforme congénita, que es una enfermedad muy rara en el gato y en el ser humano. Es el primer caso descrito en el que el motivo de consulta principal fueron los cambios de comportamiento (falta de aprendizaje de los hábitos de eliminación y de la conducta de juego).

Por otro lado, se ha visto que los problemas de comportamiento más frecuentes del perro (miedo, ansiedad por separación y agresividad) pueden alterar el perfil bioquímico y hematológico. La mayoría de los cambios no parecen inducir enfermedad de forma directa. Sin embargo, se ha observado que los perros agresivos tienen un ratio neutrófilos/linfocitos más elevado. El ratio neutrófilos/linfocitos se ha relacionado con un

mayor riesgo de afectación cardiovascular en humanos, así como con un peor pronóstico de varios problemas médicos.

Finalmente, se ha visto que la fluoxetina, uno de los fármacos más utilizados en medicina del comportamiento, también puede alterar el perfil bioquímico y hematológico del perro. Sin embargo, estos cambios no parecen ser patológicos.

Toda esta evidencia sugiere que los problemas de comportamiento y el estado de salud están íntimamente relacionados. Además, evidencia la necesidad de colaboración entre las diferentes especialidades veterinarias para mejorar en el conocimiento científico de todas ellas.

The aim of this thesis is to study the relationship between medical and behavioral problems in domestic dogs and cats. It has been divided into two main parts. The role of medical conditions on behavioral problems has been studied in the first part. The inverse relationship has been analyzed in the second part.

Animal behavior depends directly on the functioning of organs and systems. Medical conditions can modify the proper functioning of organs and systems. Thus, these medical problems can change behavior.

Through different mechanisms, pain can lead to fear, aggressive behaviors, compulsive disorders and sleep alterations. Additionally, it has been observed that there are two different patterns of expression of pain-related aggression. Dogs that were aggressive before the onset of pain may be more aggressive (more frequent and more intense) in the same context of their previous aggression and tend to be less impulsive. Otherwise, dogs that were not aggressive before the onset of pain may be more impulsive and the aggression may happen in a context of manipulation.

Behavioral changes could be the only clinical signs of many medical conditions, even without changing laboratory or imaging tests. This fact is especially important in some neurological problems that lead to behavioral changes. There are zones in the central nervous system (CNS) that have a role in the control of behavior; however, they cannot be checked by a neurological examination. Additionally, some neurological lesions of those areas may only be functional. These lesions cannot be detected in the neurological examination nor in the laboratory or imaging tests. Finally, neurological problems that lead to changes in the neurological examination and behavioral problems, but without having changes in the work-up can exist. This thesis shows one of these cases, a congenital spongiform polioencephalomyolopathy in a cat. This is a very strange disease in cats and human beings, and it is the first case where the main complaint of the owners were the behavioral changes (learning impairment regarding elimination habits and play behavior).

On the other hand, it has been observed that most common behavioral problems (fear, separation anxiety and aggressive behaviors) can lead to changes in the biochemical and hematological panel. Most of these changes do not directly lead to medical problems. Nevertheless, it has been demonstrated that aggressive dogs show a higher neutrophils/lymphocytes ratio. The neutrophils/lymphocytes ratio has been related to a higher risk of developing cardiovascular problems, and to a poor prognosis of several medical conditions.

Finally, it has also been observed that fluoxetine, one of the most common drugs used in behavioral medicine, can modify the biochemical and hematological panel of dogs. However, these changes do not seem to be pathological.

All of this evidence suggests that behavioral and health problems are intimately related. Additionally, they highlight the importance to collaborate among the different veterinary specialities in order to improve the scientific knowledge of all disciplines.

INTRODUCCIÓN GENERAL

1. DEFINICIONES

La etología clínica es la especialidad veterinaria que se encarga del estudio de los problemas de comportamiento de los animales de compañía. Un problema de comportamiento puede definirse de diferentes maneras. Sin embargo, todas las definiciones tienen tres elementos en común: (1) que pueden ser peligrosas para las personas u otros animales, (2) peligrosas para el propio animal que las lleva a cabo o, por último, (3) molestas para los propietarios.

Las mascotas pueden realizar conductas que son peligrosas para las personas o para los animales. El ejemplo más ilustrativo son las conductas agresivas. Otras conductas pueden ser peligrosas de forma más indirecta como, por ejemplo, la falta de control durante el paseo (podría tirar a una persona al suelo y hacerle daño) o una conducta de juego descontrolada (mordiscos o arañazos durante el juego). Sin embargo, que una determinada conducta sea más o menos peligrosa, no depende únicamente de la conducta en sí. Por ejemplo, si un perro muestra agresividad hacia los propietarios cuando estos intentan quitarle la comida, esta conducta será más o menos peligrosa dependiendo del tamaño y raza del perro, de la edad de la persona que intente quitarle la comida, de si la persona tiene algún problema médico (p.ej. inmunosupresión, *diabetes mellitus*, problemas de coagulación, etc.) y de si la conducta es previsible y, por lo tanto, puede evitarse. En otras palabras, la peligrosidad de una conducta no viene determinada únicamente por el tipo de conducta en sí, sino que es variable en función de otros factores.

El segundo elemento para definir un problema de comportamiento es que algunas conductas pueden ser peligrosas para el propio animal. De nuevo, pueden serlo de forma directa e indirecta. Las automutilaciones que se producen como consecuencia de un exceso de la conducta de acicalamiento, podrían ser un ejemplo de una conducta que es perjudicial de forma directa para el propio animal. Pero, además, algunas de estas conductas que se repiten de forma constante –conductas compulsivas- impiden que el animal pueda llevar a cabo otras conductas que son importantes para él (como, por ejemplo, el juego, el contacto social con otros individuos, la conducta de ingesta, etc.). Además, muchos de los problemas de comportamiento conllevan una respuesta de estrés asociada (problemas de miedo, fobia, agresividad, ansiedad por separación, etc.). El estrés, tanto agudo como crónico, puede tener consecuencias importantes para la salud del animal. La anorexia por estrés en los gatos es un problema que requiere tratamiento urgente, ya que podría terminar con la vida del animal (Center et al., 1993) y que puede

ser provocado por un estrés agudo (Dimski and Joseph, 1995). Por otro lado, está muy documentado que el estrés crónico en el gato puede favorecer la aparición de problemas urinarios de las vías bajas (Buffington et al., 2006; Kruger and Osborne, 2009; Defauw et al., 2011).

Finalmente, el factor más importante para que una conducta sea considerada por los propietarios como un problema de comportamiento es que sea molesta. Este elemento de subjetividad es muy importante. Si la conducta del animal no es percibida como molesta por los propietarios no buscarán ayuda veterinaria y, por lo tanto, no será diagnosticada. En este caso, una conducta que puede ser peligrosa para las personas y/o perjudicial para el propio animal no será tratada.

2. APROXIMACIÓN AL DIAGNÓSTICO Y TRATAMIENTO DE LOS PROBLEMAS DE CONDUCTA

La visita de etología debe recoger la información de todos aquellos factores que pueden influir sobre el comportamiento. La genética del animal (Scott y Fuller, 1965: Hart y Hart 1985; Mc Cune 1995; Ramos et al., 1997; Marchei et al., 2001; Boissy et al., 2005; Takeuchi et al., 2005; Ogata et al., 2006; Murani et al., 2010; Mehrkam LR y Wynne CDL 2014), las experiencias durante las fases del desarrollo (primeros meses de vida)(Scott 1958; Allen y Haggett 1977; Crump y Chevins, 1989; Poltyrev et al., 1996; Ward et al., 2000; Dickerson et al., 2005), las experiencias en la edad adulta y el estado de salud están entre estos factores.

2.1. Salud y comportamiento

El hecho de que un animal se comporte de una determinada manera depende, de forma directa, de cómo perciba el entorno y de cómo procese la información. Por lo tanto, necesitará del correcto funcionamiento de los órganos sensoriales (para percibir la información del entorno y de el mismo), de los diferentes órganos encargados de procesar la información (SNC) y de los órganos encargados de poner en marcha la conducta (sistema músculo esquelético, corazón, pulmones, etc.). Si algunos de estos órganos o sistemas tiene un mal funcionamiento, la respuesta de conducta que podrá dar el animal será diferente. Es decir, el estado de salud del animal tiene un papel fundamental en su comportamiento.

Esta relación es bidireccional. Está ampliamente estudiado en medicina humana que los problemas de salud mental pueden modificar la salud física del paciente. Sin embargo, aunque hay evidencias de que los problemas de comportamiento de los animales también

pueden afectar a su salud (p.ej. el estrés como factor desencadenante de la cistitis idiopática) (Buffington et al., 2006; Kruger and Osborne, 2009; Defauw et al., 2011), esta dirección de la relación está mucho menos estudiada en medicina veterinaria (Mills et al., 2014).

2.2. La visita de etología clínica

La visita se dividirá en dos grandes partes. En la primera parte, la entrevista con el/los propietario/s, se recogerán todos los datos referentes a los períodos del desarrollo, al comportamiento de la ascendencia, de los hermanos y de la descendencia (si fuera el caso), a las circunstancias del problema en sí, a las condiciones ambientales en las que vive el animal, a los tratamientos intentados (experiencias previas) y, finalmente, se realiza un historial médico. La segunda parte tiene como objetivo descartar patologías médicas que pueda causar o empeorar el problema. Siempre deberá incluir, al menos, un examen físico general (EGF), un examen neurológico, un hemograma completo y un perfil bioquímico que será más o menos amplio en función del problema. Todas estas pruebas son de coste razonable y pueden orientar el diagnóstico.

Con todos estos datos se obtiene una sospecha de si el origen del problema es etológico, médico o mixto. En función de la sospecha se establecerá el diagnóstico diferencial y el protocolo diagnóstico a seguir a partir de ese momento.

Llegados a este punto quizás sean necesarias pruebas más costosas y/o más invasivas con el fin de llegar a un diagnóstico definitivo.

El tratamiento de los problemas de comportamiento se puede dividir en tres grandes grupos de medidas (Bowen y Heath 2005; Beaver 2009; Hernández 2012; Overall 2013b).

En primer lugar, las técnicas de modificación de conducta. Hacen referencia a aquellas técnicas que, mediante el aprendizaje, intentan cambiar la conducta del animal. Suelen ser la base del tratamiento de la mayoría de los problemas de conducta.

En segundo lugar, la castración está indicada para aquellas conductas que son sexualmente dimórficas (aquellas conductas que son más frecuentes en un sexo)(Hopkins et al., 1976; Neilson et al., 1997).

Finalmente, el uso de psicofármacos, feromonas y nutracéuticos como coadyuvantes de las técnicas de modificación de conducta está indicado para numerosos problemas de comportamiento. Dos de los objetivos más comunes a la hora de usar psicofármacos es reducir la ansiedad (ansiolisis) y disminuir la agresividad. Actualmente la fluoxetina es el

fármaco más usado para ambos fines. Es un fármaco ampliamente estudiado en medicina humana y, en menor medida, en medicina veterinaria. Tiene como objetivo aumentar la actividad de la serotonina como neurotransmisor en el SNC. Es un fármaco de uso crónico. En la mayoría de las ocasiones debe tomarse durante meses (de hecho tarda unas 2-6 semanas en empezar a hacer efecto), años o, incluso, durante toda la vida del animal (Beaver 2009; Hernández 2012).

Los semioquímicos son moléculas muy volátiles que transportan un mensaje. Las feromonas son un tipo de semioquímico. Las feromonas se liberan al entorno desde diferentes complejos de liberación localizados en la superficie corporal (complejo podal, facial, mamario, etc.). Una vez liberadas, se difunden por el medio desde donde son percibidas (por un proceso mixto activo/pasivo) por otros individuos (normalmente de la misma especie). Cuando son percibidas, las feromonas producen un cambio de comportamiento en el receptor (Pageat y Gaultier 2003). Existen multitud de aplicaciones clínicas de diferentes feromonas sintetizadas tanto en el perro (*Dog Appeasing Pheromone*-D.A.P.) como en el gato (F3 *Fraction of the Cat Facial Pheromone*; F4 *Fraction of the Cat Facial Pheromone-Cat Allomarking Pheromone* and *Feline Interdigital Semiochemical*-FIS) (Bowen y Heath 2005; Beaver 2009; Hernández 2012; Overall 2013b).

Finalmente, los nutracéuticos son nutrientes aislados, suplementos dietéticos, productos herbales o alimentos que tienen propiedades terapéuticas. Los más usados en veterinaria son la aplha-casozepina que es un derivado lácteo con propiedades ansiolíticas (son potenciadores del GABA-como las benzodiacepinas), los productos antioxidantes (usados especialmente para enlentecer el envejecimiento cerebral), y los precursores de nuerotransmisores (p.ej., el triptófano como precursor de la serotonina)(Milgram et al., 2004; Head et al., 2009; Overall 2013c).

3. IMPORTANCIA DEL ESTUDIO DE LOS PROBLEMAS DE COMPORTAMIENTO

Como se ha visto en el primer apartado, la propia definición de "*problemas de comportamiento"* incluye factores que son subjetivos. Esto puede conllevar, erróneamente, a menospreciar la importancia que tienen dichos problemas. Sin embargo, las consecuencias de estos comportamientos sí son objetivas. El estudio de los problemas de comportamiento es importantes por cuatro motivos: (1) su frecuencia, (2) sus consecuencias sobre la salud pública, (3) sus consecuencias económicas y, finalmente, (4) sus efectos sobre el bienestar de los animales.

3.1. Frecuencia

Los estudios basados en encuestas dirigidas a propietarios de animales de compañía son los más usados para determinar la prevalencia de los problemas de comportamiento. Con independencia de dónde estén hechos, la mayoría de estudios demuestran que los problemas de conducta en el perro y el gato son muy frecuentes. La mayoría de estos estudios toman como criterio de inclusión, para considerar que el animal tiene un problema de conducta, el hecho de que la conducta sea molesta para los propietarios. Uno de los primeros estudios llevados a cabo (Voith, 1985) muestra que el 40% de los propietarios (de EEUU) encuestados dicen que su perro o gato hace con frecuencia una o más conductas molestas. En otro estudio, también llevado a cabo en EEUU, el 87% de los perros mostraban, al menos, una conducta molesta (Campbell, 1986). La media de comportamientos problemáticos por perro era de 4.7. En Europa, un estudio realizado en Gran Bretaña (n=50) encontró que el 80% de los perros mostraban alguna conducta molesta (O'Farrell, 1992). Una encuesta no publicada, hecha también en Gran Bretaña y que incluyó 772 perros de 502 hogares, muestra que el 76% de los perros habían mostrado en alguna ocasión agresividad, el 70% eliminación inadecuada, el 57% tiraban de la correa y el 48% se excitaban con facilidad (Lindell, 2002).

Finalmente, un estudio realizado en España mediante encuestas a veterinarios no especialistas (n=433), muestra que 98.3% de estos son consultados por problemas de comportamiento (Fatjó et al., 2006).

En cuánto a la distribución de los problemas, la mayoría de los estudios epidemiológicos muestran divergencias en función de si las encuestas se realizan en centros de referencia (especialistas en etología clínica) o en clínicas no especializadas. En el caso de los perros, la agresividad es el problema más visitado por veterinarios etólogos seguida, de lejos, por problemas de vocalización, eliminación y destructividad (lo que engloba los tres signos clínicos más típicos de la ansiedad por separación)(Borchelt and Voith, 1996; Lindsay, 2001; Base de datos de la *Fundació Hospital Clínica Veterinari* de la UAB). En el caso de veterinarios no especialistas, estos 4 síntomas siguen siendo los más frecuentes, pero no pueden diferenciarse estadísticamente entre ellos (Fatjó et al., 2006). En el caso de los gatos, tanto en veterinarios especialistas como en centros generalistas, los problemas de eliminación inadecuada son los más visitados, entre el 40% y el 50% del total de los casos en función del la base de datos (Overall, 1997; Fatjó et al., 2002a). La agresividad es el segundo motivo de queja más frecuente, de nuevo, en ambos casos (entre un 30% y un 35% de los casos visitados)(Overall, 1997; Fatjó et al., 2002a). Sin embargo, un estudio muestra el 54% de los gatos bufan a las personas con frecuencia (entre una vez al mes y

una vez a la semana), y el 60% arañan o muerden ocasionalmente a la gente (Borchelt y Voith 1987).

En resumen de estos datos se extraen tres ideas. Primero, que los problemas de comportamiento son muy frecuentes. Segundo, que los veterinarios suelen ser consultados como medio para resolver dichos problemas. Tercero, que los problemas de agresividad son muy frecuentes tanto en perros como en gatos.

3.2. Salud pública

Los problemas de conducta pueden tener un impacto sobre la salud pública. La incidencia de accidentes por mordedura en EEUU dirigidos hacia personas se estima entre 500.000 y 4.5 millones accidentes/año (Sacks et al., 1996a; Quinlan et al., 1999) aunque parece que la cifra está más cerca de los 5 millones. El ratio se sitúa en 15,8 mordeduras por cada 1000 personas y año (Gilchrist et al., 2008). En Francia entre 150.000 y 500.000 por año (Chomel et al., 1992) y en el Reino Unido 230.000 anuales (Thomas et al., 1990). Uno de estos estudios (Sacks et al., 1996a) estima que el número de ataques mortales por año en los EEUU es de 20, lo que supone un ratio de muertos por año de 7.1 por cada 100 millones de personas (Sacks et al., 1996a; Langley 2009). Un estudio (Weiss et al., 1998) apunta a que la incidencia de mordeduras por perros adopta una distribución en forma piramidal, y estima que por cada ataque mortal que se produce en EEUU, 670 personas deben ser hospitalizadas por mordeduras de perros, 16.000 más serán atendidas en centros de urgencias sin requerir hospitalización, 21.000 acudirán a otros centros médicos (no de urgencias) y 187.000 más serán mordidas sin necesitar o demandar asistencia médica. Algunos datos disponibles en países europeos no difieren de los datos expuestos hasta el momento. Por ejemplo, según la Agencia Estatal de Estadística Holandesa, que maneja la base de datos que registra las causas de muerte de todos los residentes en Holanda, la media de muertes por año en Holanda entre 1996 y 2006 causadas por ataques de perros fue de 9.1 por 100 millones de personas (incluso superior a la registrada en EEUU). La mayoría de las mordeduras son producidas por el perro de la familia (Horisberg et al., 2004; De Kuester et al., 2006).

Pero todos estos datos adquieren una importancia mayor si se tienen en cuenta las características de los principales grupos a riesgo dentro de la población. Las principales víctimas de las mordeduras caninas son los niños (Berzon et al., 1972; Morton 1973; Underman 1987; Gershman et al., 1994; Sacks et al., 1996b; Patrick et al., 1998; Ozanne-Smith et al., 2001; Rosado et al., 2009). Se estima que los niños tienen una probabilidad de entre 3 y 5 veces superior que una persona adulta a ser mordido por un perro (Sacks et al.,

1996b; Overall y Love 2001). En España los estudios realizados muestran datos similares a los encontrados en la bibliografía, apuntando a que los niños menores de 15 años presentan un riesgo significativamente mayor a ser mordidos que el resto de grupo de edades (Rosado et al., 2009). En EEUU se estima que más del 50% de los niños a la edad de 12 años habrán sido mordidos en algún momento de su vida por un perro (Spiegel, 2000). El ratio de niños mordidos en Bélgica por año es de 22 por cada 1000 (De Kuester et al., 2006). Además la tasa de mortalidad en neonatos y bebés es significativamente superior a la de los adultos (Sacks et al., 1996a).

Las razones por las cuales los niños tienen un riesgo mayor a ser mordidos por perros son varias. Entre las causas más importantes se encuentra la menor capacidad por parte de los niños para identificar las señales de alarma que da el perro antes de llegar a morder (Mathews et al., 1994).

En cuánto a las causas de por qué tienen más probabilidad de morir como consecuencia de estos ataques encontramos su menor habilidad para evitar los ataques y su menor capacidad de defensa frente a estos (Chun et al., 1982). Además, tienden más a relacionarse con los perros abrazándolos y cogiéndolos de la cabeza (Mathews et al., 1994). Esta característica, además de por la estatura, podría ser la razón de por qué la mayoría de las mordeduras en niños se localizan en la cara y en los brazos. Entre los niños de 0 a 9 años el 73 % de las mordeduras se localizan en la cabeza y el cuello mientras que en el resto de grupos de edad sólo el 30 % de las mordeduras tienen esta distribución (cuello y cabeza) (Weiss et al., 1998). Aunque los porcentajes son menores, otro estudio más reciente hecho en los Países Bajos (Cornelissen et al., 2009) corrobora estos datos (un 31 % de las heridas en niños se localizarían en la región craneal y cuello frente al 8 % de los adultos). Las mordeduras localizadas en estas zonas del cuerpo tienen un mayor riesgo de ser fatales que las que se localizan en otras zonas como las extremidades inferiores.

Por otro lado, los arañazos de gatos son los responsables de la zoonosis más frecuente inducida por los animales de compañía, la enfermedad del arañazo del gato (Macías et al., 2014). Afecta a unas 24.000 personas al año en EEUU, de las cuales unas 2000 requieren hospitalización (Windsor 2001). La incidencia real es difícil de establecer porque no es de declaración obligatoria, pero algunos estudios estiman que se movería entre los 0.77 and 0.86 por 100 000 personas al año (Jackson et al., 1993). De nuevo, el principal grupo de riesgo son los niños (el 55% de los casos de dan en personas de menos de 18 años). Es una enfermedad estacional que tiene un pico de incidencia entre setiembre y enero (el 60% de los casos se concentran en esta época del año)(Jackson et al., 1993).

En resumen, los problemas de conducta de los perros y gatos, en especial la agresividad, representan un riesgo para la salud pública.

3.3. Coste económico

En los EEUU el gasto sanitario derivado de este tipo de accidentes asciende entre 170 millones y 1000 millones de dólares por año (Overall and Love, 2001). En EEUU también tienen un coste elevado las reclamaciones de los trabajadores a sus aseguradoras, alrededor de 10 millones de dólares al año (Beaver, 2009). El total de las reclamaciones a los seguros de hogar ascienden a cerca de 1000 millones de dólares anuales (Beaver et al., 2001).

Los problemas de conducta son una de las principales razones de abandono. El coste del mantenimiento (con servicios básicos) de cada animal abandonado asciende a 2200 dólares por gato y 3300 dólares por perro (de media por todo el tiempo de permanencia media en los centros de recogida de EEUU) (Overall, 2013a).

El coste anual estimado en estados unidos de la enfermedad del arañazo del gato (sanidad) es de más 12 millones de dólares (Jackson et al., 1993).

3.4. Bienestar animal

Los problemas de comportamiento pueden tener un impacto sobre el bienestar de los animales de compañía por varias razones.

En primer lugar, porque son una de las principales razones de abandono y eutanasia de los animales de compañía. De los 10 a 17 millones de perros que entran en los centros de acogida en EEUU, del 36% al 59% no tienen propietario. Del resto, sobre un 30% son llevados por sus propietarios al centro (Beaver 2009). Los problemas de comportamiento son la principal causa de abandono en estos casos (46.4% de los casos)(Scarlett et al., 1999). Cada año se eutanasian entre 15 y 20 millones de perros solo en refugios en EEUU (Overall 1997). De estos, el 70% se deben a problemas de comportamiento (Beaver 2009). Estas cifras son un buen reflejo de la importancia que tienen los problemas de conducta, sobre todo, si se tiene en cuenta que en estos datos solo está incluido un país (EEUU), y solo los datos de refugios (no hay datos del número de perros eutanasiados en centros privados por la misma causa).

En el caso de los gatos, los problemas de comportamiento son la segunda causa de abandono, solo por detrás de las alergias de los propietarios. Son la primera causa de

abandono debido a un factor del animal (por delante de cualquier problema de salud)(Scarlett et al., 1999).

Finalmente, un estudio demuestra que tanto los perros como los gatos tienen, proporcionalmente, más probabilidades de ser abandonados cuánto más "ensucian la casa", cuántas más "cosas rompan" y cuánto "más activos" sean (New Jr et al., 2000). Curiosamente, solo los perros (no los gatos) tienen más riego de ser abandonados si han mordido en algún momento (New Jr et al., 2000).

Los problemas de comportamiento pueden alterar el bienestar del animal por otros motivos. Aunque existen muchas definiciones de bienestar, el *Farm Animal Welfare Council (FAWC)*, un comité de expertos que asesora al gobierno británico en términos de bienestar, unificó todas esas definiciones en el principio de las cinco libertades. Según el FAWC, para asegurar el bienestar de los animales de renta (aunque es extrapolable a cualquier animal) se deben cumplir, al menos, los siguientes requisitos:

1. Ausencia de sed y hambre prolongada (*Freedom from hunger and thirst*).
2. Ausencia de incomodidad física o térmica (*Freedom from discomfort*).
3. Ausencia de dolor, lesiones o enfermedad (*Freedom from pain, injury or disease*).
4. Libertad para expresar su comportamiento normal (*Freedom to express normal behaviour*).
5. Ausencia de miedo o estrés (entendido como estrés negativo para el animal) (*Freedom from fear and distress*).

Muchos problemas de comportamiento comprometen uno o más puntos de las cinco libertades. Las dos primeras "libertades" guardan poca relación con la etología clínica. La cuarta y la quinta "libertad" están directamente relacionadas con los problemas de comportamiento.

Muchos problemas de conducta ponen en marcha una respuesta de estrés en el animal (p.ej., problemas de miedos y fobias, de agresividad, de ansiedad por separación, etc.). Cuando estos problemas no son tratados y se cronifican son, por si solos, indicadores de falta de bienestar del animal.

Algunos problemas de comportamiento impiden que el animal pueda llevar a cabo el propio comportamiento normal de la especie. Un ejemplo claro son los trastornos compulsivos. Los trastornos compulsivos son conductas que se repiten durante mucho tiempo pudiendo, incluso, llegar a interferir con las conductas normales del animal.

Además, algunas de estas conductas compulsivas pueden llegar a producir lesiones importantes en el propio individuo (p.ej. la dermatitis acral por lamido), lo que enlazaría con la tercera "libertad".

La tercera "libertad", *a priori*, guarda poca relación con los problemas de comportamiento. Sin embargo, está ampliamente documentado, por ejemplo, que el principal signo clínico del dolor es el cambio en el comportamiento del animal (Hellyer et al., 2007). Además, como se ha mencionado anteriormente, algunos problemas de comportamiento pueden alterar el estado de salud del propio animal. Sin embargo, este hecho, que está ampliamente demostrado en medicina humana, está poco estudiado en medicina veterinaria (Mills et al., 2014)

En definitiva, parece existir una relación entre los problemas de comportamiento, el estado de saludo del animal y su bienestar.

4. ESTRÉS Y SALUD

Existen muchas definiciones de estrés. Todas ellas tienen varios factores en común: (1) es una respuesta fisiológica que se da en (2) respuesta de un estímulo que, de forma real o potencial, amenaza la homestasis del animal. (3) La respuesta de estrés es necesaria para la supervivencia del animal y para su adaptación al medio. (4) En función de diferentes factores puede tener un efecto positivo, neutral o nocivo (incluso mortal) para el animal (Trevisi y Bertoni 2009). (5) Entre estos factores se encuentran, entre otros, la duración del estímulo desencadentante, su intensidad y predictibilidad o control sobre el mismo, las experiencias previas del animal con ese estímulo y las diferencias individuales en la expresión de la respuesta de estrés (Manser 1992). Los cambios fisiológicos que se ponen en marcha en la respuesta de estrés (ver más adelante) conlleva cambios en el comportamiento. Estos cambios de conducta, que se definen clásicamente como de huída o lucha, tienen como finalidad resolver la situación estresante.

Desde el punto de vista fisiológico la respuesta de estrés conlleva la activación del eje Hipotálamo-Pituitario-Adrenal. Se inicia con la liberación de la hormona liberadora de corticotropina o CRH. Tanto la respuesta fisiológica como de comportamiento de la respuesta de estrés son consecuencia de las acciones de la CRH. Entre los efectos más destacados de la respuesta de estrés se encuentran: (1) como consecuencia de la liberación de catecolaminas se produce taquicardia, taquipnea, hipertensión, vasoconstricción periférica, dilatación bronquial, aumento del metabolismo energético (hiperglucemia) e hipertermia y, (2) Como consecuencia de la acción de glucocorticoides se observa gluconeogénesis, proteólisis, movilización de grasas, actividad antiinflamatoria,

reducción de la sensibilidad del dolor e inmunosupresión (Manteca 2009). Como se ha comentado anteriormente, todos estos cambios tienen como objetivo preparar al animal para huir o luchar o, en otras palabras, para hacer frente a la situación estresante.

La respuesta fisiológica de estrés supone un coste biológico para el animal en términos de inmunidad, gasto de energía, etc. En términos generales, cuando la respuesta de estrés se ajusta en tiempo e intensidad al estímulo estresante no suele acarrear problemas para el animal, ya que este dispone de suficientes reservas biológicas. En otras palabras, aunque con excepciones, la respuesta de estrés aguda no suele acarrear un coste biológico elevado para el animal y suele ser beneficiosa en términos adaptativos. Sin embargo, la respuesta de estrés se cronifica cuando, a pesar de los intentos repetidos, el animal no consigue adaptarse al entorno estresante. En estos casos es más probable que las reservas biológicas se agoten. Este agotamiento supone un coste para el animal que, normalmente, se traduce en problemas de salud y alteraciones del comportamiento.

No todos los animales tienen la misma respuesta de estrés ante los mismo estímulos o entornos estresantes. Existen "diferencias individuales" en la respuesta de estrés que, además, se traducen en diferencias individuales en la expresión del comportamiento (Manteca y Deag, 1993; Koolhas 2008). Es decir, no todos los animales, aunque sean de la misma especie, se comportan de la misma manera ante una situación dada.

Como se acaba de ver no todos los animales responden de la misma manera ante los mismos estímulos estresantes. Cuando más ajustada sea la respuesta de estrés, en términos de duración e intensidad, menor coste biológico tendrá para el animal. Los períodos del desarrollo juegan un papel fundamental en el desarrollo de una correcta respuesta de estrés.

Finalmente, la respuesta de estrés no depende únicamente de la naturaleza o la intensidad del estímulo estresante, sino que también depende de cómo el animal percibe dicho estímulo. En términos generales se ha visto que cuánto más predecible es un estímulo, menos estresante es para el animal (Weinberg y Levine 1980; Sapolsky 2004; Lovallo 2005) y, por lo tanto, menos coste biológico tendrá. Durante las situaciones estresantes el animal puede realizar asociaciones entre diferentes estímulos para, en un futuro, poder predecirlos mejor. De hecho, la capacidad de aprendizaje se ve incrementada inicialmente en la respuesta de estrés (Manteca 2009).

4.1. Estrés y períodos del desarrollo

Período prenatal

El estrés de la madre durante la gestación puede producir cambios permanentes en la descendencia. Estos efectos han sido ampliamente estudiados en numerosas especies como, por ejemplo, en ratones (Allen y Haggett 1977; Crump y Chevins, 1989), en ratas (Poltyrev et al., 1996; Ward et al., 2000; Dickerson et al., 2005), en cobayas (Sachser y Kaiser 1995; Kaiser y Sachser 1998; Kaiser y Sachser 2001), en monos ardilla (Schneider y Coe 1993; Coe y Crispen 2000) y en niños (Meijer 1985; Glover 2011) entre otras. Se han estudiado tanto estímulos estresantes sociales como no sociales. Se ha visto que las consecuencias sobre el comportamiento son muy variadas, y varían en función de la especie y del estresante usado en el estudio. Entre los cambios de comportamiento descritos están, entre otros, la masculinización, la feminización del comportamiento sexual, una menor capacidad para resolver problemas de orientación y el comportamiento infantil permanente. También se han descrito cambios hormonales y de neurotransmisores, tales como el aumento de la testosterona sérica (Kaiser y Sachser 1998; Kaiser y Sachser 2001) y la disminución de la actividad de serotonina (Hayashi et al., 1998). Sin embargo, desde un punto de vista práctico, el efecto más importante del estrés prenatal sobre el comportamiento de la descendencia, es que promueve un comportamiento más ansioso cuando son expuestos a ambientes estresantes (Poltyrev et al., 1996; Ward et al., 2000; Dickerson et al., 2005).

Aunque no hay estudios en perros y gatos es probable que, dados los estudios en otras especies, el estrés prenatal pueda tener un efecto sobre el comportamiento adulto de la descendencia similar al descrito arriba.

Período neonatal

Las experiencias durante los primeros días después del nacimiento (período neonatal) también tienen un impacto permanente sobre el comportamiento adulto del cachorro. Este fenómeno es especialmente importante en aquellas especies más inmaduras en el momento del nacimiento (especies altriciales). En perros y gatos el período prenatal comprende, aproximadamente, las dos primeras semanas de vida (Manteca, 2003).

Los primeros estudios se realizaron en roedores de laboratorio. Los investigadores separaban a las crías de las madres durante un corto período de tiempo (5 – 15 minutos). Al ser devueltas con la madre, esta incrementaba el lamido sobre las crías que habían sido separadas. Este incremento del lamido, y consecuentemente de estimulación táctil, parece

ser el responsable de cambios epigenéticos que favorecen la adaptación a ambientes estresantes cuando la cría es adulta (Levine 1957; Levine 1962; Levine et al., 1962: Priestnall 1973; Lay 2000; Meaney 2001).

Se han demostrado efectos similares en los perros. Perros que eran manipulados (5 minutos al día) en el período neonatal, mostraban menos signos de estrés después a las 8 semanas de vida. Además, el efecto era mayor en animales que provenían de criadero en comparación con aquellos que habían estado en familia durante el período neonatal (Gazzano et al., 2008).

Los cambios neurofisiológicos inducidos por la estimulación táctil neonatal están bien estudiados. En primer lugar, existe un incremento de los receptores de glucocorticoides en el hipocampo y la corteza frontal (Meaney et al., 1985a), lo que incrementa la sensibilidad de esas estructuras al *feed back* negativo del eje Hipotálamo-Pituitario-Adrenal (HPA). Este incremento de la sensibilidad aumenta la eficacia de la inhibición neuronal sobre la secreción de hormona adenocorticotropa (ACTH)(Meaney et al., 1985b; Meaney et al., 1991). En segundo lugar, se ha observado que las ratas que son manipuladas durante su etapa neonatal retornan más rápido a los niveles basales de glucocorticoides (en concreto corticosterona) después de una situación estresante (Beane et al., 2002). Finalmente, también se ha visto que los animales manipulados tienen una menor respuesta de miedo y una menor secreción de glucocorticoides en respuesta a diferentes estímulos estresantes (Meaney et al., 1988). En otras palabras, lo que parece favorecer la manipulación neonatal es que, cuando son adultos, los animales tengan una respuesta de estrés más ajustada al evento estresante, tanto en intensidad como en duración.

Finalmente, también se ha observado que los animales manipulados durante el período neonatal tienen una mayor concentración de serotonina (Papaioannou et al., 2002). La serotonina es un neurotransmisor involucrado en el control de múltiples comportamientos. Las concentraciones bajas de serotonina se correlacionan con comportamientos agresivos, especialmente con los de tipo impulsivo. Además, muchos de los tratamientos farmacológicos utilizados para el control de la agresividad se basan en el incremento de la actividad serotoninérgica a nivel del SNC.

Período de socialización

Período de socialización contribuye de forma muy importante en las diferencias de comportamiento entre individuos. Se extiende entre la 3ª semana de vida y la 12ª-14ª en el perro, mientras que en el gato comprende entre la 2ª-3ª y la 7ª-9ª semana de vida.

El período de socialización es determinante en el comportamiento del animal adulto. La mayoría de comportamientos sociales se desarrollan, o empiezan a desarrollarse, durante el período de socialización (Lindsay 2000). Los efectos sobre el comportamiento del período de socialización son difícilmente reversibles (Manteca 2003).

Aunque todas las conductas adquiridas durante el período de socialización tienen una importancia capital en el comportamiento adulto, desde un punto de vista clínico, dos de las que tienen más relevancia son: (1) el reconocimiento de especie y (2) la habituación a estímulos ambientales.

El período de socialización se inicia cuando aparece el reflejo de amenaza y ya se han abierto los canales auditivos. Es decir, se inicia cuando el animal es capaz de poder percibir el entorno mediante todos sentidos (el gusto, olfato y el tacto están presenten, en mayor o menor medida, desde el nacimiento). Además, cuando esto sucede, los centros encargados del control de la respuesta de miedo en el Sistema Nervioso Central (SNC) todavía no están desarrollados. Al no tener miedo, el animal se muestra curioso ante multitud de estímulos del entorno. A partir de la 5ª semana la respuesta de miedo aparece y empieza a ser cada vez más intensa. Entre la 12ª y la 15ª semana ya está completamente desarrollada. A partir de este momento el animal muestra miedo a todo lo desconocido (neofobia)(Scott 1958).

Aquellos animales que son expuestos a una elevada variedad de estímulos durante el período de socialización, muestran significativamente menos miedo cuando son adultos que aquellos que han sido menos estimulados (incluso cuando se enfrentan a estímulos que nunca han visto)(Hubrecht 1995; Boxal et al., 2004). La socialización tiene un fuerte componente de reconocimiento visual de los estímulos (Manteca 2003). Un estudio demuestra que los perros que han tenido contacto con niños durante el período de socialización muestran menos probabilidad de ser agresivos o mostrar excitación en presencia de niños cuando son adultos (Arai et al., 2011). Sin embargo, el beneficio de la estimulación durante el período de socialización no es lineal. Denenberg (1964) observó que demasiada estimulación podría ser contraproducente y afectar negativamente, entre otras cosas, al aprendizaje del animal. Finalmente, los animales que son privados de

estimulación durante el período de socialización tienden a mostrar miedo extremo, hiperactividad (Thomson y Herow 1954) o inhibición extrema (Beaver 2009), disminución en la capacidad de aprendizaje (Thomson y Herow 1954) y respuestas inapropiadas a estímulos dolorosos (Melzack y Scott 1957; Markwell y Thorne, 1987).

Las pautas vacunales en cachorros suelen terminan, aproximadamente, al mismo tiempo que el período de socialización. Esto conlleva que, con frecuencia, los cachorros no sean expuestos a estímulos variados. Esta falta de estimulación hace que puedan mostrarse miedosos cuando son adultos.

Para salvar este problema existen los programas de socialización. Los objetivos de los cursos de socialización son: (1) aportar una alta variedad de estimulos a los cachorros (otras personas, perros, niños, ruidos de petardos, tormentas, etc.), en un entorno sanitariamente seguro. (2) Educar a los propietarios a prevenir los principales problemas de comportamiento de los perros. (3) Habituar al animal a las principales manipulaciones clínicas (corte de uñas, revisiones oftalmológicas, óticas, pinchazos, etc.). Los animales que pasan por cursos de socialización tienen menos probabilidades de desarrollar problemas de comportamiento cuando son adultos (Seksel 1997; Seksel 2008).

4.2. Aprendizaje y predictibilidad – las experiencas en la edad adulta

Las experiencias pueden modificar la respuesta de estrés y, por lo tanto, el comportamiento. Los animales aprenden de las experiencias. Domjan (2010) define el aprendizaje como "*un cambio duradero en los mecanismos de la conducta que involucran estímulos y/o respuestas específicos y que es resultado de la experiencia previa con esos estímulos y respuestas o con otros similares*". Es decir, cuando un animal tiene una experiencia con cierto/s estímulo/s, el aprendizaje le permite modular el comportamiento en situaciones parecidas que se puedan dar en el futuro. El aprendizaje ayuda al animal adaptarse al entorno.

Tanto en el perro como en el gato se han descrito todos los tipos básicos de aprendizaje. Es decir, tanto procesos no asociativos (habituación y sensibilización), como asociativos (condicionamiento clásico y operante)(Domjan 2010).

Como se ha señalado anteriormente, el animal puede aprender a predecir los estímulos estresantes. En este caso la percepción del mismo será menos negativa, ya que podrá controlarlo (Weinberg y Levine 1980; Sapolsky 2004; Lovallo 2005). Además, si el animal utiliza una conducta ante una situaciónn estresante y consigue solucionarla, entonces

aprenderá a utilizar la misma estrategia de conducta para resolver situaciones similares en el futuro.

5. INDICADORES BIOQUÍMICOS Y HEMATOLÓGICOS DE SALUD

Existen diferentes métodos para determinar el estado de salud de un animal. La historia clínica, los exámenes directos sobre el individuo (examen físico general, examen neurológico, etc.), los análisis de laboratorio y las pruebas de imagen, entre otros, ayudan a determinar el diagnóstico. Los análisis de laboratorio básicos sirven para encaminar el diagnóstico, son de coste razonable y poco invasivos (normalmente realizados con una muestra de sangre y/u orina). Las pruebas de laboratorio básicas suelen incluir un hemograma y una bioquímica sanguínea (que podrá incluir diferentes parámetros). La información debe ser tenida en cuenta como un todo a la hora de llegar a un diagnóstico definitivo.

5.1. Hemograma

El hemograma tiene como objetivo describir la cantidad y calidad de las células sanguíneas (Weiss y Tvedten 2004). Hoy en día los métodos automáticos son los más usados en la clínica diaria. Sin embargo, siempre se aconseja realizar un frotis sanguíneo y, en algunos casos, un examen de la médula ósea.

Según describen Weiss y Tvedten (2004) la evaluación de los resultados de un hemograma debe hacer, sistemáticamente, siguiendo tres pasos: (1) Identificar los resultados anómalos, y usar la terminología correcta para describirlos. A la hora de determinar los resultados anómalos, se debe tener en cuenta algunas excepciones que pueden existir dependiendo de la raza. Por ejemplo, los perros de la raza St. Bernardo pueden tener un hematocrito algo por debajo del límite bajo sin ser patológico, y al revés puede suceder con los galgos. El segundo punto es, (2) Evaluar las anormalidades encontradas en el hemograma teniendo en cuenta todos los datos del mismo, es decir, evaluar el hemograma de forma global (normalmente incluyendo hallazgos del frotis). Por ejemplo, si el hematocrito está bajo y se sospecha de una anemia, deberá definirse el tipo de anemia teniendo en cuenta el recuento de reticulocitos y la morfología de los glóbulos rojos (frotis). Finalmente, (3) Obtener las conclusiones, un diagnóstico de laboratorio. Los parámetros incluidos en el hemograma se resumen en la tabla 1:

Parámetro	Información
Hematocrito	Representa el porcentaje del volumen total de la sangre que está compuesta por lo glóbulos rojos. Al ser un porcentaje de un volumen del total sanguíneo depende del número de glóbulos rojos, de su tamaño y del porcentaje de plasma sanguíneo.
Hemoglobina	Concentración de hemoglobina.
RBC	Es el número total de glóbulos rojos.
WBC	Es el número total de glóbulos blancos. El hemograma también informa, además del WBC, del porcentaje y del número total de cada tipo de glóbulo blanco: Neutrófilos en Banda, polimorfonucleares neutrófilos, linfocitos, monocitos, eosinófilos y basófilos.
Plaquetas	Es el número total de plaquetas.
MCV	Indica el tamaño medio de los glóbulos rojos.
MCH	Es la masa de la hemoglobina contenida en un glóbulo rojo. Sirve para determinar si una anemia es hipo o hipercrómica.
MCHC	Es la concentración de hemoglobina en un volumen determinado de glóbulos rojos. Clínicamente la MCH y la MCHC son medidas redundantes.

Tabla 1. Parámetros incluidos en el hemograma. RBC (*Red Blood Cell Count*); WBC (*White Blood Cell Count*); MCV (Volumen Corpuscular Medio); MCH (Hemoglobina Corpuscular Media); MCHC (Concentración Corpuscular Media de Hemoglobina).

5.2. Bioquímica sérica

La función básica es determinar el funcionamiento de los diferentes órganos del animal y, en algunos casos, encaminar o definir un diagnóstico. Es una prueba muy poco invasiva ya que se necesita, únicamente, una muestra sanguínea. Dependiendo de los parámetros que se vayan a determinar se deberán tomar algunas precauciones en la toma de la muestra. Por ejemplo, si se quiere determinar la glucosa, el suero o plasma deberá ser separado lo antes posible ya que, en caso contrario, las células contenidas en la sangre continúan consumiendo glucosa y se pueden obtener parámetros falsamente bajos (Nelson et al., 2004).

Existen muchos parámetros bioquímicos que aportan información sobre la estructura o función de los diferentes órganos. Los parámetros más comúnmente analizados desde un

punto de vista clínico son: Alanina transaminasa o alanina aminotransferasa (ALT), aspartato aminotransferasa (AST), fosfatasa alcalina (ALKP), gamma-glutamil transpeptidasa (GGT), Ácidos biliares, bilirrubina total, colesterol, triglicéridos, calcio total, cloro, fósforo, sodio, potasio, creatinina, urea, creatin-kinasa (CK), glucosa, fructosamina, T4 total y la tirotropina (TSH).

Resulta difícil clasificar los diferentes parámetros según las posibles alteraciones orgánicas ya que muchos de ellos pueden verse alterados por varias enfermedades. Sin embargo, en términos generales, las enzimas ALT, AST, ALKP y GGT son indicativas de daño celular hepático. Algunas son más especificas en determinadas especies o patologías. Por ejemplo, la ALKP parece ser más sensible de enfermedad hepatobiliar en el perro que la GGT. Por el contrario, la GGT es algo más específica en gatos de enfermedad hepática (salvo para lipidosis hepática)(Willard y Twedt 2004). Otros parámetros, sin embargo, se usan para determinar la función hepática (a diferencia de los anteriores que valoran la integridad celular únicamente), como por ejemplo, el test de los ácidos biliares o, incluso, la concentración de urea (un parámetro valorado típicamente para función renal) o la concentración de albúmina puesto que ambos se sintetizan en el hígado.

La urea y la creatinina se usan para determinar la función renal. Conjuntamente con la concentración de proteínas y algunos electrolitos como el fósforo, pueden dar una idea de cuál es la funcionalidad de los riñones.

Los electrolitos pueden verse modificados por multitud de patologías. Corregir rápidamente sus desequilibrios lo antes posible suele ser más beneficioso para el paciente que el propio diagnóstico aunque, lógicamente, las dos cosas son deseadas.

Algunas alteraciones de comportamiento se han asociado a alteraciones hormonales. Por ejemplo, tanto el hipo como el hipertiroidismo (Seibert y Landsberg 2008) se han relacionado con agresividad, hiperactividad y reacciones de miedo entre otras (Fatjó et al., 2002b; Beaver y Haug 2003). La determinación de las hormonas tiroideas (TSH y T4 total) son necesarias para el diagnóstico de algunas de estas patologías. Además, otros datos del la bioquímica sérica, como los triglicéridos y, especialmente, el colesterol, pueden ser de gran utilidad para definir el diagnóstico.

Alteraciones endocrinas que cursan con poliuria y polidipsia pueden confundirse con alteraciones de comportamiento que cursan con cambios en los hábitos higiénicos del animal. El hiperadrenocorticismo o la diabetes mellitus serían un buen ejemplo. La

concentración de glucosa, fructosamina o de algunos electrolitos, pueden ayudar a diagnosticar el problema y a instaurar el tratamiento más indicado.

5.3. Proteinograma

Las proteínas séricas pueden verse alteradas por muchas patologías. Pueden ser indicativas de infecciones víricas, infecciones bacterianas, parasitosis, enfermedades autoinmunes entre muchas otras. Algunos fármacos también pueden modificar (como los corticoides o las hormonas tiroideas) (Werner et al., 2004).

A parte de la concentración de proteínas totales, el proteinograma incluye las concentraciones de albúmina, globulinas totales, α1- globulinas, α2- globulinas, β-globulinas y las γ-globulinas.

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OBJETIVOS

OBJETIVOS

El objetivo general de esta tesis es evaluar la relación entre los problemas médicos y los problemas de comportamiento en el perro y el gato doméstico y su relación con los parámetros bioquímicos y hematológicos.

Estos objetivos generales se pueden dividir en los siguientes objetivos específicos:

1. Describir los principales problemas médicos del perro y el gato que pueden cursar con cambios de comportamiento, y que suponen un reto diagnóstico por la dificultad a la hora de diferenciarlos de un problema de conducta puro (capítulos 1 y 2).
2. Evaluar el papel del dolor en la expresión de la agresividad en el perro (capítulo 3).
3. Evaluar el efecto de los problemas de comportamiento sobre los parámetros hematológicos y bioquímicos del perro (capítulo 4).
4. Evaluar el efecto de la fluoxetina sobre los parámetros hematológicos y bioquímicos del perro (capítulo 5).

CAPÍTULO 1

Medical conditions and behavioral problems in dogs and cats. An article review.

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ABSTRACT

Not all animals behave identically when faced with the same situation. These individual differences in the expression of their behavior could be due to many factors, including medical conditions. These medical problems can change behavior directly or indirectly. The aims of this review are to describe the state of the art of the relationship among some medical and behavioral problems, and to propose new lines of investigation. The revision is focused on the relation between behavioral problems and pain, endocrine diseases, neurological problems, vomeronasal organ alterations, and cardiac disorders. These problems represent a diagnostic challenge from a practical point of view. The most common sign of pain in animals is a change in behavior. Although the relation of pain to behavioral problems has been widely studied, it is not absolutely clear. As an example, the relation between sleep disorders and pain is poorly known in veterinary medicine. New studies in human beings and laboratory animals show that a reciprocal relationship does, in fact, exist. More specifically, the literature suggests that the temporal effect of sleep deprivation on pain may be stronger than that of pain on sleep. Some behavioral problems could modify the sleep-awake cycle (e.g. cognitive dysfunction). The impact of these behavioral problems on pain perception is completely unknown in dogs and cats. Thyroid hormones play an important role, regarding behavioral control. Both hypothyroidism and hyperthyroidism have been related to behavioral changes. Concerning hypothyroidism, this relationship remains controversial. Nonetheless, new neuro-imaging studies provide objective evidence that brain structure and function are altered in hypothyroid patients, both in laboratory animals and in human beings. There are many neurological problems that could potentially change behavior. This paper reviews those neurological problems that could lead to behavioral changes without modifying neurological examination. The most common problems are tumors that affect central nervous system silent zones, mild traumatic brain injury, ischemic attacks and epilepsy. Most of these diseases and their relationship to behavior are poorly studied in dogs and cats. To better understand the pathophysiology of all of these problems, and their relation to behavioral problems, may change the diagnostic protocol of behavioral problems.

1. INTRODUCTION

Not all animals behave identically when faced with the same situation. These individual differences in the expression of their behavior could be due to many factors. Genetics, pre-natal manipulation of the bitch (Kaiser and Sachser, 2005; Weinstock, 2008), experiences of the animal during the different developmental stages (especially important, neonatal and socialization periods) (Scott, 1962), experiences during adulthood and the correct functioning of organs and systems are among these factors.

Medical conditions can modify the proper functioning of organs and systems. Thus, these medical problems can change behavior directly or indirectly. These problems could be divided into four main groups: Problems that modify or prevent the perception of the environment (e.g. blindness), problems that change the processing of the perceived information (e.g. intracranial tumor) or alter the internal processes (hormonal and/or neurological) involved in behavior (e.g. hypothyroidism), problems that induce a stress response that can modify behavior (e.g. pain) and problems that change or prevent the expression of behavior (e.g. a broken leg).

The relationship between medical and behavioral conditions has been widely investigated, but many doubts still exist in that regard.

The aims of this review are to describe the state of the art of the relationship between some medical and behavioral problems, and proposing new lines of investigation.

2. MATERIAL AND METHODS

Problems included in this revision have been those that can contribute to lead to behavioral problems, but which are difficult to differentiate from a true behavioral or medical problem. We have included those problems that represent a diagnostic challenge from a practical point of view.

The first review has been performed using Pubmed and Google Scholar. References cited in the articles found in the first review have also been taken into account.

3. PAIN AND BEHAVIORAL PROBLEMS

The most common sign of pain in animals is a change in behavior (Hellyer et al., 2007). Behavioral signs of pain include both the loss of normal behaviors and the development of new and abnormal behaviors. The most common behaviors classified as “lost normal behaviors” are decreased ambulation or activity, lethargic attitude and decreased appetite and decreased resting behaviors. The most common “developed abnormal behaviors” are

aggression, fear reactions, inappropriate elimination, vocalization, decreased interaction with other pets or family members, altered facial expression, altered posture, restlessness and hiding (Hellyer et al., 2007). Pain has also been related to repetitive behaviors (Asmundson and Katz, 2009).

From a clinical point of view, pain should be included in the differential diagnoses of aggressive behaviors, fear and phobia reactions, sleep disorders, inappropriate elimination, vocalization and repetitive behaviors. However, according to the authors' experience, pain-related vocalizations, as a main complaint of the owner, are very unlikely.

3.1. Pain and aggressive behaviors

It is widely accepted that pain can lead to aggressive behaviors that are often described as a defensive reaction to avoid physical contact that may cause further injury (Rutherford, 2002). Moreover, the animal could learn about a painful experience (Fanselow, 1980). If it has experimented pain in a specific context, it may avoid the same or a similar context in the future, displaying passive behaviors (e.g. fear and phobias) or more reactive ones (e.g. fear-related aggressive behaviors), even when the animal is not yet in pain.

However, it also well-described that pain elicits a stress response (Mellor et al., 2000). Pain-induced responses lead to many physiological and behavioral changes. Decreased serotonin activity in the brain would be among them (Mellor et al., 2000). Moreover, pain could also decrease physical activity, and this may further reduce serotonin activity in the CNS (Chaouloff, 1997). Finally, a reduction of serotonin activity in the CNS has been related to aggressive behaviors (Tsatsoulis et al., 2006).

It could be that these two different mechanisms may result in a different pattern of aggressive behavior caused by pain. In fact, a study based on 12 clinical cases (Camps et al, 2011) showed that two different patterns of pain-related aggression exist, depending on whether the dog had been aggressive before the onset of pain. According to this clinical case-study, dogs that were aggressive before the onset of pain may be more aggressive (more frequent and more intense) in the same context of their previous aggression and tend to be less impulsive. Otherwise, dogs that were not aggressive before the onset of pain may be more impulsive and the aggression may happen in a context of manipulation. In other words, this second group seems to display avoiding behaviors, whereas the first group may decrease the aggression threshold as a response to pain.

3.2. Pain and fears

Pain also can induce fear. First, pain acts as an unconditional stimulus, which induces a fear response (Brown, 1951; Meulders et al., 2011). Therefore, when an animal is in a situation in which it experiences pain, it will try to create associations between the stimulus that causes pain and other neutral stimuli (Fanselow, 1980; Meulders et al., 2015). These neutral stimuli may help to predict a similar situation in the future. When the conditional context is present again, the animal may show fear even in the absence of the initial unconditional stimulus.

A second mechanism exists that is demonstrated in human beings but not in animals. It is well-known that people and animals that are in pain, especially a chronic one, are more likely to suffer from anxiety (Asmundson and Katz, 2009; Lyons et al., 2015). Due to this anxiety state, these people have a higher probability of showing a pessimistic cognitive bias, which makes initially neutral stimuli become potential (unreal) sources of pain, which may lead to more anxiety and fear (Dehghani et al., 2004). However, a similar process could also happen in animals. A clinical-case report of two dogs with fear towards unconventional stimuli was presented (Lindley, 2012). These dogs did not respond to conventional treatment of fear. After months, the animals were also diagnosed with pain. Subsequently, the dogs were treated simultaneously for fear and pain, and the problem was solved.

It is well-know, that pain can also lead to anxiety in dogs (Mathews, 2000). Moreover, dogs with another source of anxiety (separation anxiety) exhibit a pessimistic cognitive bias (Mendl et al., 2010). It may be that animals with pain can suffer from anxiety, and that anxiety leads to a pessimistic cognitive bias that also leads to fear reactions in dogs. Further studies are needed in order to confirm this hypothesis.

3.3. Pain and sleep disorders

It is widely accepted that pain and sleep are related. However, many questions remain about the direction of causality. Brain structures associated with the generation and maintenance of sleep are also involved in pain modulation, providing a neurobiological substrate for a reciprocal relationship (Smith and Haythornthwaite, 2004). Early longitudinal and experimental evidence reviewed by many authors (Smith and Haythornthwaite, 2004; Doufas et al., 2012; Finan et al., 2013) in the human-being literature revealed that a reciprocal relationship exists, where pain results in sleep disturbance and disturbed sleep enhances pain. It seems that acute pain can lead to a night of poor sleep in a human patient who had no previous sleep complaints, with effects that

are usually reversible (Lavigne et al., 2011). In the case of chronic pain this relationship is more controversial. In fact, a trend in the literature suggests that the temporal effect of sleep deprivation on pain may be stronger than that of pain on sleep (Finan et al., 2013). In other words, insomnia symptoms significantly increase the risk of developing future chronic pain disorders in previously pain-free individuals, whereas existing pain is not a strong predictor of new incident cases of sleep disturbances. Additionally, good sleep increases the chance that chronic pain will improve over time.

There is a lack of studies in dogs and cats regarding this issue. Nevertheless, it could be interesting to investigate it. First, it may be a good model for human beings medicine. Secondly, behavioral problems in small animals that lead to sleep alterations are common, above all, in elderly patients (cognitive dysfunction syndrome) (Neilson et al., 2001). It may be interesting to investigate how sleep alterations can modify the pain threshold in those animals in order to guarantee their welfare.

3.4. Pain and inappropriate elimination

As has been mentioned before, pain acts as an unconditional stimulus, which induces a fear response (Brown, 1951; Fanselow, 1980). Feline urinary tract disease is common in cats, and leads to pain in most cases (Kruger and Osborne, 2009). Therefore, if a cat is in pain when urinating, it can make a relation between pain and the litter-tray. In other words, pain is a common cause of litter-tray aversion in cats. A similar process may happen with feces and problems that induce pain during defecation (Bowen and Heath, 2005; Overall, 2013).

3.5. Pain and Repetitive behaviors

Pain and compulsive disorders are related. In human beings, individuals who suffer from chronic pain show more anxiety disorders than does general population (35% against 17%) (Asmundson and Katz, 2009). Anxiety disorders includes obsessive-compulsive disorders, among others. As an example, women with fibromyalgia are four to five times more likely to have had a lifetime diagnosis of obsessive-compulsive disorder, post-traumatic stress syndrome, or generalized anxiety (Raphael et al., 2006).

There is a lack of specific studies about the real role of pain on compulsive disorders in animals. However, two mechanisms could be involved. First, it is widely accepted that stress plays an important role in the development of compulsive behaviors in animals as well as in human beings. Pain elicits a stress response in animals (Mellor et al., 2000), and could act as a trigger of a compulsive behavior by itself, as a source of stress. Secondly,

pain may lead to a licking behavior directed toward the painful area of the body. Then, this licking behavior could be reinforced by itself because it decreases the sensation of pain (Melzack and Wall, 1965), and by the owners because they often pay attention to the animal when it performs the behavior (operant conditioning). A good example could be the licking behavior directed toward the caudal area of the abdomen in cats with bladder pain.

4. ENDOCRINE DISEASES AND BEHAVIORAL PROBLEMS

Thyroid hormones play an important role regarding behavioral control. In fact, the brain is a major target organ for thyroid hormones (Samuels, 2014). Thyroid hormone alterations have been associated with behavioral problems in animals as well as human beings. However, some authors think that causality of the relationship is unlikely (Overall, 2003) and defend that the problems may be just co-morbid and co-exist in time. Nevertheless, some new data strongly suggest that a causative relationship could exist. New imaging studies provide objective evidence that brain structure and function are altered in hypothyroid patients, with decreased hippocampal volume, cerebral blood flow and function globally, and in regions that mediate attention, working memory and motor speed in human being and in rats (Constant et al., 2001; Lass et al., 2008; Bauer et al., 2009; He et al., 2011; Cooke et al., 2014). Moreover, a recent study (He et al., 2011) showed that some of these alterations (alterations in working memory and abnormalities in functional magnetic resonance) were no longer present after six months of treatment with levothyroxine. Finally, thyroid hormones have been found to affect the turnover of serotonin (Mason et al., 1987, Henley et al., 1991). Serotonin is involved in the control of behavior (e.g. aggression and fear) (Müller and Jacobs, 2010).

Similar studies in dogs do not exist. However, in clinical reports, hypothyroidism has been classically associated with aggression, and also to apathy, lethargy or mental dullness, cold intolerance, exercise intolerance and decreased libido (Beaver and Haug, 2003). Additionally, treatment with levothyroxine improves clinical signs, including aggressive behaviors (Fatjó el al., 2002). However, some authors argue that levothyroxine can change behavior by itself, including normal and euthyroid animals (Seibert and Landsberg, 2008). For this reason, and in the absence of double-blind control studies, the link remains speculative for some authors (Overall, 2003; Seibert and Landsberg, 2008).

In human medicine, as a summary, overt hypothyroidism (elevated serum thyroid-stimulating hormone –TSH-, and low, free-thyroxin level) is associated with clinically significant neuropsychiatric decrements that are largely reversible with levothyroxine

treatment, whereas mild or subclinical (elevated TSH with normal, free thyroxin) is not associated with severe or widespread neuropsychiatric decrements (Samuels, 2014). In this case, if neuropsychiatric symptoms are present, they usually are unrelated to the thyroid problem, and these do not reliably reverse with thyroid supplementation. Veterinary medicine has a similar approach. In all dogs where a behavioral problem co-exists (especially important aggressive and fear-related behaviors) and an overt hypothyroidism, the supplementation is recommended and often solves or improves the behavioral problem. However, in euthyroid dogs (having low total T4 and/or free T4, with TSH within the normal values and other pathologies or with concomitant use of other drugs) the treatment with levothyroxine is not recommended, in spite of having a behavioral problem and low serum concentration of T4 (Overall, 2003). In these cases, it is unlikely that the behavioral problem and the thyroid alterations are in relation.

Hyperthyroidism has been associated with behavioral changes in animals and in human beings. It is well-known that hyperthyroidism has a major effect on the development of the nervous system (Legrand, 1986; Porterfield, 1993; Oppenheimer and Schwartz, 1997; Chan, 2000; Bernal, 2002; Anderson et al., 2003; Morreale, 2004; Ahmed et al., 2008; Ahmed, 2015). Hyperthyroidism during developmental phases may result in an irreversible impairment, morphological abnormalities, disorganization and cytoarchitectural abnormalities (Ahmed et al., 2008). These changes would be permanent. However, from a clinical point of view, and although a causative relationship has not been proven, a relationship between hyperthyroidism in cats and aggression has been suggested (Seibert and Landsberg, 2008). Hyperthyroidism in cats is not typically a developmental disease. In fact, it is a disease of geriatric cats with a mean age of 13 years (Scott-Moncrieff, 2012). Nevertheless, it is known that intraperitoneal administration of T3 and T4 (for seven days) increased the number of cortical beta-adrenergic and serotoninergic receptors (Mason et al,. 1987) in adult rats. Additionally, recent data (Hassan et al., 2013) suggest that iatrogenic hyperthyroidism in adult rats also changes the levels of dopamine, norepinephrine and serotonin in different brain regions as well as in blood plasma, cardiac muscle and the adrenal glands. All of these neurotransmitters have an important role in behavioral control. Thus, this relationship may explain, at least in part, behavioral changes seen in adult and elderly animals with hyperthyroidism. On the other hand, other authors suggest that this relationship occurs only as a co-morbid condition (Overall, 2003).

5. NEUROLOGY AND BEHAVIORAL PROBLEMS

CNS is directly involved in the control of behavior. Thus, there are many neurological problems that could potentially change behavior. The behavioral changes due to neurological issues could be divided into four subgroups. First, animals that show behavioral changes, changes in the neurological examination and changes in the laboratory and/or imaging work-up. Second, animals that have behavioral changes and changes in the neurological examination, but without having changes in the work-up. Third, animals that have behavioral changes without having neurological changes, but that show work-up alterations. Finally, there are animals that show behavioral changes due to neurological alterations, but without changing neurological and laboratory or imaging work-up.

Problems of Group One and Two are not discussed here because they are problems easy to differentiate from behavioral problems, and are commonly diagnosed and treated by neurologists.

There are areas in the CNS that are silent to the neurological examination (Foster et al., 1988). In human beings, frontal and prefrontal cortexes are two of those areas. Frontal lobes have been identified experimentally as silent zones in animals (Kandell et al., 2000). Some of these areas have a major role in behavioral control. Animals that have lesions (e.g. tumors, ischemic lesions, etc.) in those areas could show behavioral changes (and/or seizures) without having changes in neurological examinations. One retrospective study (Foster et al., 1988) that included 43 dogs with tumors that affected the rostral brain showed that 22 dogs had seizures alone as their initial sign, four dogs had seizures and behavioral changes upon initial examination and five dogs had shown abnormal behavior patterns only. Additionally, 31 of the 43 dogs had a normal neurological examination upon initial presentation. However, 25 of these 31 dogs developed persistent neurological deficits later. Importantly, eight dogs never developed neurological deficits and were euthanized because of uncontrolled seizures or unacceptable behavior. It is important to realize that in this study the age of the dogs ranged from 5 to 15 years, which does not overlap with the mean age of onset of behavioral problems (≈12-24 months of age) (Overall, 2003). Thus, in practical terms, the age of onset of the problem could be used in these animals as an indicator that more invasive laboratory and imaging work-up is justified.

Finally, some neurological problems change the behavior without changing neurological examination nor imaging nor laboratory tests. All of these problems have been described

in human beings, and some of them also in laboratory and companion animals. The most common problems in this group are: idiopathic epilepsy, mild traumatic brain injury and transient ischemic attacks.

Seizures are the most common neurological problem reported in dogs that are owned as pets (Podell et al., 1995). Seizures result from abnormal electrical discharges in the brain. The most commonly given diagnosis for canine seizures is idiopathic epilepsy (Ghormley et al., 2015). There are different classifications in the veterinary literature for seizures. Nonetheless, empirical studies have systematically classified canine seizures as generalized and partial or focal (Licht et al., 2002). In fact, partial seizure with secondary generalization seems to be the seizure type most observed in dogs (Podell et al., 1995; Heynold et al., 1997; Berendt and Gram, 1999). Generalized as well as partial seizures have been related to behavioral changes in dogs and cats.

Generalized seizures are caused by abnormal electrical discharges in large areas of the brain. In human beings, several and an increasing number of studies have identified a relationship between psychiatric disorders and recurrent seizures disorders (Jalava and Sillanpaa, 1996; Gaitatzis et al, 2004; Tellez-Zenteno et al., 2005; Nuyen et al., 2006; Austin and Caplan, 2007; La France et al., 2008). This co-morbidity has also been reported in laboratory rats (Heinrichs and Seyfried 2006; Gastens et al, 2008) and companion dogs (Shihab et al, 2011). Depression and anxiety disorders followed by psychoses and attention deficit disorders are the most common psychiatric disorders in human medicine. Similarly, Shihab and colleagues showed that dogs with epilepsy have a higher risk to display fear/anxiety-type behaviors and defensive aggression, and show abnormal perception (that included barking without apparent cause, chasing shadows or lights, aimless pacing, and staring into space). Interestingly, this relationship seems to be bi-directional. People with a history of depression or suicide could have up to a seven-times-greater risk of developing epilepsy (Hesdorffer et al., 2006; La france et al., 2008).

On the other hand, in partial seizures, caused by localized abnormal discharge, the clinical signs depend on the affected area of the brain. Thus, partial seizures have been associated with different behavioral alterations both in human beings and animals. The human ILAE (International League Against Epilepsy) classification of partial seizures has been used as a model of classification of animal partial seizures (Berendt et al., 2004). Berendt et al. (2004) studied 70 dogs, with a confirmed diagnosis of epilepsy with partial seizures with or without secondary generalization. They recorded the signs of partial seizure activity following the human ILAE classification, and found that 80% of dogs (n=56) showed

paroxysms of behavior, 69% of dogs (n=48) showed motor signs, and autonomic signs were recorded in 23% of dogs (n=16). Importantly, nine dogs (13%) had partial seizures without secondary generalization. The majority of dogs showed a combination of signs of, at least, two groups of signs. However, a pool of dogs could exist with behavioral changes due to partial seizures without any other evident neurological sign seen by the owner. Additionally, these dogs might not show any abnormality during the neurological examination. Finally, in 48 dogs (69%), the first seizure was observed before or at three years of age (Berendt et al., 2004), which overlaps with the mean age of onset of behavioral problems (≅ 12 – 24 months)(Overall, 2003). All of these highlight the difficulty of the diagnosis and treatment of those animals, above all, considering that some drugs used in behavioral medicine could decrease the epileptic threshold (Stokes and Holtz, 1997).

Traumatic injury is a common occurrence in veterinary medicine (Simpson et al., 2009), and traumatic brain injury occurs in a high proportion of these animals (Fletcher and Dewey, 2009). A single-center retrospective study reported that up to 25% of dogs with severe blunt-force trauma suffer from traumatic brain injury (Simpson et al., 2009). TBI is defined as structural injury or physiological disruption of the brain induced by an external force (Beltran et al., 2014). Although dogs with TBI are assessed clinically using the modified Glasgow coma scale (GCS), there is no standardized classification of severity for TBI in animals. There is an index of disease severity called improved survival prediction index (SPI2), but it is not specific for trauma patients, and relies on the most severe values for the first 24-hour period after the admission to the ICU service (King et al., 2001). Nevertheless, in human-being medicine, TBI is classified into three different categories of severity: mild, moderate and severe, depending on GCS along with four more values (Structural imaging, loss of consciousness, alteration of consciousness/mental state and post-traumatic amnesia).

There are studies in human medicine with mild TBI (mTBI) that highlight changes in conventional clinical neuro-imaging (MRI scanning or computed tomography (CT)). However, these changes are present in a low percentage of people. Only 5% to 10% of people with mild TBI showed abnormalities in CT (Williams et al., 1990; Borczuk, 1995; Miller et al., 1997; Haydel et al., 2000). Nevertheless, thanks to studies in human beings and experimental animal models, it is well-known that mTBI leads to diffuse axonal injury because of biochemical forces and a host of injury-mediated cytotoxic processes (Arciniegas et al., 2005). Additionally, acute and chronic alterations of neurotransmitter production and/or delivery have been associated with mTBI (Povlishock, 1992;

Obrenovitch and Urenjak, 1997; Arciniegas, 2003). Most of these alterations are visible using functional neuro-imaging techniques. They more accurately reflect the extent of damaged tissue than either conventional CT or MRI (Arciniegas et al., 2005). Studies with both single photon computed tomography (SPECT) and positron emission tomography (PET) suggest that TBI leads to disturbances in brain function even when there are no abnormalities in conventional neuro-imaging techniques (CT and MRI) (Choksey et al., 1991; Mitchener et al., 1997). The use of these techniques in veterinary medicine as a diagnostic tool is anecdotal.

Importantly, the vast majority of people recover fully during the first year following mTBI (Arciniegas et al., 2005). However, from 1% to 20% of people who have suffered from TBI will develop persistent cognitive, emotional, behavioral, and/or physical impairments that extend for more than one year following the TBI. The most common cognitive, emotional or behavioral changes are irritability, depression, apathy, impulsiveness, and attention and/or memory impairments (Arciniegas et al., 2005). There is a lack of studies in veterinary medicine about the impact of TBI on behavior. Nevertheless, it could be interesting to study this event in veterinary medicine due to two reasons. First, as we previously mentioned, because traumatic injury is common in veterinary medicine (Simpson et al., 2009), and TBI occurs in a high proportion of those animals (Fletcher and Dewey, 2009). A better understanding of this pathology in animals may help us to improve their quality of life. Second, animals could be used as models for human medicine, and may be useful to improve the diagnoses and treatments of animals and human beings.

Similarly to mTBI, transient ischemic attacks (TIA) can lead to behavioral problems in human beings without showing changes in the neurological examination nor conventional neuro-imaging techniques (MRI and CT). In fact, from 20% to 50% of TIAs do not show MRI abnormalities (Brazzelli et al., 2014). As occurs with TBI, the lack of visible lesions using MRI does not rule out the presence of microscopic and functional damage that can change the behavior. Recent studies, where rats were used as models for TIAs, reveal that a similar process could also happen in animals (Ejaz et al., 2015). Transient iatrogenic occlusion of the middle cerebral artery in rats leads to selective neuronal loss and microglial activation both in striatum (Li et al., 1999; Sicard et al., 2006) and the cerebral cortex (Ejaz et al., 2015), despite a normal MRI. Loss of normal function of these areas, especially of the cerebral cortex, may lead to long-term behavioral abnormalities.

All of these problems are a diagnostic challenge due to the absence of consistent imaging changes or other neurological signs.

6. VOMERONASAL ORGAN AND BEHAVIORAL PROBLEMS

Pheromones may be defined as a kind of semiochemical freed from the external surfaces of the body, from where they diffuse into the surrounding environment and change the behavior of the other animals (normally of the same species). Pheromones are perceived by the vomeronasal organ (VNO). The VNO is a part of the accessory olfactory tract, and it is located on each side of the nasal septum (Pageat and Gaultier, 2003). Post-mortem studies in different species show that VNO can be affected by inflammatory changes (Verin et al., 2010; Asproni et al., 2014). Inflammatory changes may alter the function of any tissue. Thus, inflammatory changes of VNO may lead to behavioral alterations. Verin et al. (2010) suggest that vomeronasalitis in cats could be related to aggressive behaviors in cats. It could be interesting to investigate the possibility to detect these inflammatory changes *in vivo*, in order to improve the early detection and the treatment of the problem.

7. CARDIOLOGY AND BEHAVIORAL PROBLEMS

Cardiac problems may lead to behavioral problems, especially because these problems can decrease the physical activity of the animal. However, it is not common that those problems are referred to a behaviorist. And they usually improve after cardiac treatment.

However, cognitive dysfunction is a problem commonly treated as a behavioral problem that could be in close relation to cardiac problems. Cognitive dysfunction in animals has been typically treated as a mitochondrial malfunction that leads to neuronal oxidative damage. Nevertheless, this oxidative damage could be due to a reduced or altered blood perfusion of the CNS, and cardiac problems may be the cause of this reduced perfusion. This relation is well established in human medicine (Gorelick 2004; Paul et al., 2005). Epidemiological studies reveal that 61% of the people with dementia have Alzheimer disease (AD), 54% have cardiac problems, and vascular dementia (VaD) and AD are present together in the majority of people (Victoroff et al., 1995; Snowdon et al., 1997; MRC CFAS, 2001; Lange and Paul et al., 2005). However, although in human beings the real prevalence of VaD remains unclear, most of the studies rank VaD as the third most common type of severe dementia in the elderly (Lange and Paul, 2005). Among people with dementia associated with VaD, life expectancy is significantly shortened, when compared to the general population (Wolfson et al., 2001). Although the quality of life (QoL) of these patients has not been widely studied, some authors suggest that decreased QoL in those patients could be significant (Paul et al., 2005). The prevalence and physiopathology of this problem has not been studied in animals. Nonetheless, its study could be interesting because animals may be used as animal models for human medicine

in order to improve the QoL of animals and persons. In fact, some of the vascular risk factors that have also been linked to dementia in human beings (hypertension, obesity, high levels of triglycerides, low levels of HDL and diabetes among others) (Nash and Fillit, 2006) are present in dogs and cats too. Some of those risk factors are present, even in apparently healthy middle-aged and old animals (Paepe et al., 2013). Thus, the study of VaD should be taken into account in future research in veterinary medicine.

8. CONCLUSIONS

This revision illustrates the relation between the development of many behavioral problems and different medical conditions. Many of these medical conditions have, as only clinical signs, a behavioral change. Additionally, some of them do not change any hematological or imaging parameter, which makes their diagnoses and their differentiation from a true behavioral problem more difficult. All of this illustrates the importance of taking into account the medical problems in the differential diagnoses of any behavioral problem, and the significance of doing a thorough follow-up all of the cases.

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CAPÍTULO 2

A case of spongiform polioencephalomyelopathy in a cat with a history of behavioural problems

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ABSTRACT

A 7-month-old, entire female, domestic shorthair cat was referred to our behavioural service due to house-soiling and a play-related problem. The two owners' complaints were that the cat had never used the litter tray, and she did not know how to play. After reviewing her behavioural history, a problem of substrate-preferences acquisition was suspected, regarding the elimination problem. During the consultation, the physical examination was unremarkable, but the neurological examination revealed a moderate and hypermetric ataxic gait, and a bilateral lack of menace response. Some degree of visual impairment was suspected. The problem was located in the CNS, and specifically an intracranial and multifocal problem was diagnosed. After a complete work-up (complete ophthalmologic examination, CBC and a complete biochemistry panel, FIV/FeLV test, thorax X-ray, abdominal ultrasound, brain MRI (0.2 T), CSF analysis and a urinary metabolic screen test), a degenerative problem of CNS was suspected. No treatment was prescribed for the neurological problem. Regarding the house-soiling problem, reward-based training with a clicker was used, and she partially improved in a few weeks. Three months later, the cat was referred to the neurology service in *status epilepticus*. A symptomatic treatment was prescribed, with a mild response. After two years of treatment and a progressive worsening, the cat was euthanized. The necropsy revealed a diagnosis of spongiform polioencephalomyelopathy. In order to rule out prion aetiology a PrPsc inmunohistochemistry assay was performed, and the results were negative. Congenital spongiform polioencephalomyelopathy was diagnosed. We strongly suggest that her behavioural clinical signs were caused by the congenital spongiform polioencephalomyelopathy, causing learning impairment. To the best of our knowledge, this would be the first case where a congenital degenerative disease affects the cat's capability to learn and leads to behavioural signs as the main complaint of the owners, even before neurological signs were detected by the owners.

A 7-month-old, entire female, domestic shorthair cat was referred to our behavioural service due to house-soiling and a play-related problem. The two owners' complaints were that the cat had never used the litter tray, and she did not know how to play. The environment consisted of two young adult humans, with no children. They lived in a flat of 85 m^2, with two terraces of 5 m^2 each. There were three separated litter-boxes at home. All of them were non-covered with low sides. The owners had used clumping, non-clumping, silica-based, and soil-based litter during the months between the adoption (when the cat was four months old) and the first visit. One of the latrines always had clumping substrate. There were three food and three water troughs, all of them far from the latrines.

The impression of the owners was that the cat eliminated where she was at any given moment. She eliminated many more times in front of the owner (90%) than being alone (10%). The cat never tried to cover her faeces or urine after depositions. Occasionally, they had punished the cat verbally and physically, but only when she eliminated in front of them. The substrates used by the cat were ceramic tiles, the sofa and beds. She always adopted an emptying body posture. Spots were always located on horizontal surfaces. The owners used bleach-based products in order to clean the spots, and just water in the case of the sofa and beds.

Regarding the play-related problem, the owners said that she did not understand the body language of the other cats and, commonly, crashed into the other cats or people. She also "tried to bite, catch and scratch the air" when playing. She did not find balls or other toys when they threw them to her to play.

The rest of her behavioural history was unremarkable.

The differential diagnoses of the house-soiling problem should include: first problems with the litter trays, including insufficient number, incorrect type, competition with other cats for the latrines, incorrect location, acquired aversion, or inappropriate substrate. Secondly, there may be a preference for another location or substrate. Third, there could be a problem with preference acquisition (i.e. because of a cognitive impairment, or a sensory impairment or an unavailability of appropriate latrines or substrate during the first few weeks of life). Fourth, marking behaviours. Finally, a medical illness can contribute to all of these problems or to be the main cause[1,2].

We could rule out most of these problems after interviewing the owners. First, it was unlikely that there was a problem with the litter tray because the number, type and location were correct. The locations were correct because the animal eliminated near the

litter tray if she was there. Many different substrates had been used. Secondly, it was not a problem of preference because the cat eliminated in different locations and surfaces. The age of the animal, the distribution of the spots, and the body posture during elimination ruled out marking behaviours.

Alterations in play behaviours described by the owners could be due to a cognitive impairment, and/or a sensory impairment (i.e. blindness). Play behaviours depend on learning capability and sensory systems[3]. Additionally, an enriched environment is necessary to learn and display play behaviours in a proper manner[4]. In that case, the social and instrumental environment was good.

Regarding elimination, a problem of substrate preferences acquisition was diagnosed. A cognitive impairment, a medical condition, or both, could have been the cause of the problem during the elimination-habits acquisition. Moreover, cognitive impairment and some medical conditions also could explain the play-related behavioural problems.

During the consultation, the physical examination was unremarkable, but the neurological examination revealed a moderate and hypermetric ataxic gait, and a bilateral lack of menace response. Then, a complete ophthalmologic examination was done by the ophthalmological service in order to rule out ocular diseases. No ophthalmologic abnormalities were detected. Additionally, based on the behaviours at home described by the owners (the inability to find some toys, and the behaviour of "scratching and biting the air"), some degree of visual impairment was suspected but not confirmed with the neurological examination. The cat did not crash with objects either at home or at the consultation room. Then, the problem was located in the CNS, and specifically an intracranial and multifocal problem was diagnosed. A CBC and a complete biochemistry panel were performed, and all of the results were within normal limits. The FIV/FeLV test was negative. A thorax X-ray, abdominal ultrasound, brain MRI (0.2 T) and CSF analysis showed no abnormalities. Although some small lesions could be missed with low field MRI, we had to assume the absence of lesions obtained in our work-up. Thus, a degenerative condition such as a lysosomal storage disease, organic aciduria, or mitochondrial encephalopathy, was suspected. Samples of blood and urine were sent to the University of Pennsylvania School of Veterinary Medicine (3900 Delancey Street, Philadelphia, PA 19104-6010) for metabolic screen tests. Amino acids, organic acids, carbohydrates, nitroprusside, ketone, and mucopolysaccharide concentrations were analysed, as well as alpha-mannosidase, beta-mannosidase, fucosidase, and hexosaminidase A & B activity. All of these were within normal limits.

No treatment was prescribed for the neurological problem. The owner was given advice to correct the house-soiling problem using reward-based training with a clicker. During the first week, the clicker was conditioned by a food reward, and the entire floor was covered with newspaper. Each time that the cat eliminated, it was rewarded with a clicker and food. Newspaper was removed progressively. Three months later, the cat used a small newspaper-covered area to eliminate. This partial improvement suggests that there was a learning impairment during the acquisition of habits, but not a total lack of learning capability.

After three months, the cat was referred to the neurology service again in *status epilepticus*. Neurological findings, after post-ictal phase, included a lack of bilateral menace response and cerebellar ataxic gait. A bilateral carpal valgus that had already been found in the first visit, and a visible suture line in the posterior capsule of both crystalline lenses were also detected. The owners reported progressive gait deficits over the previous month and compulsive running episodes with a partially impaired mental status (probably seizure activity). A symptomatic treatment with diazepam (1 mg/Kg intra-rectal only if seizures) and phenobarbital (2 mg/Kg PO SID) was started, with a very poor response. After 15 days of treatment, levetiracetam was added (10 mg/Kg PO TID) due to an increase in seizure activity, with an initially good response. However, seizures reappeared after two months with 1-2 episodes every 15 days each lasting less than two min. After two years of treatment and a progressive worsening, the cat was euthanized. A complete necropsy was immediately performed. No gross lesions were found, and based on the CNS histological lesions shown in Figures 1, 2, 3 and 4, a diagnosis of spongiform polioencephalomyelopathy was made. The spongiform degeneration of the grey matter was extensively distributed in the whole CNS. In order to rule out prion aetiology, a PrPsc inmunohistochemistry assay was performed, and the results were negative. Thus, congenital spongiform polioencephalomyelopathy was diagnosed (*post-mortem*).

Spongy vacuolation seen by light microscopy in the neural tissue is defined as spongy degeneration, and may take the form of vacuoles within processes of the neuropil, vesiculation of myelin sheaths, or swelling of astrocyte or oligodendrocyte cytoplasm[5]. A congenital problem[6,7,8], retrovirus infection[9] and prion disease[10] have been suggested as possible aetiologies. Congenital spongiform degeneration of the grey matter has been previously described in a few cases of cats[6,7,8]. The cause remains still unknown. Common clinical signs include gait alteration, seizures, blindness, bilateral cataracts, behavioural changes and cranial nerve alterations. These signs appear during very early stages of development (just after birth), with a progressive fatal outcome in a few days or months.

Behavioural signs are poorly described in animal science literature[6,7,8]. Although the degeneration usually affects diffusely all the grey matter, the behavioural alterations, and the evolution of the clinical signs, depend on the affected area of the brain in each case. Nevertheless, the clinical signs do not always correlate with the degree of the histological lesions. A spongy degenerative problem of grey matter has also been described in human beings, and occurs in isolated cases and in sibs[11,12,13]. In all of human cases reported, the problem appears early in infancy, and the outcome is always fatal. All the affected children show learning disabilities (i.e. retarded speech development) during the early periods of infancy. Additionally, they rapidly develop neurological signs, especially seizures. The clinical findings and neurophatological changes are very similar in both human beings and the present case. There are no studies regarding degeneration of grey matter and its effect on learning ability in animals. However, other neurodegenerative problems (i.e. lysosomal storage diseases) and problems that lead to structural abnormalities of the forebrain (i.e. hydrocephaly), may be correlated with learning disabilities in animals and human beings[14,15,16,17].

The acquisition of the elimination habits occurs during the first weeks of kittens' life. Most of the kittens naturally seek out sand-like materials for elimination purposes. However, the preference for a substrate needs to be learnt during those first weeks of age. Learning disabilities and/or sensory impairments could modify the acquisition of these habits.

In conclusion, we strongly suggest that the behavioural signs (elimination and the play-related problem) were caused by congenital spongiform polioencephalomyelopathy, causing learning impairment.

In conclusion, this case contributes to scientific knowledge with two interesting facts. First, it describes a congenital spongiform polioencephalomyelopathy, a very rare condition described in cats. Secondly, to the best of our knowledge, this would be the first case where a congenital degenerative disease affects the cat's capability to learn and leads to behavioural signs as the main complaint of the owners, even before neurological signs were detected by the owner.

Finally, this case illustrates the importance of considering medical conditions in all behavioural cases and of using an accurate diagnostic protocol.

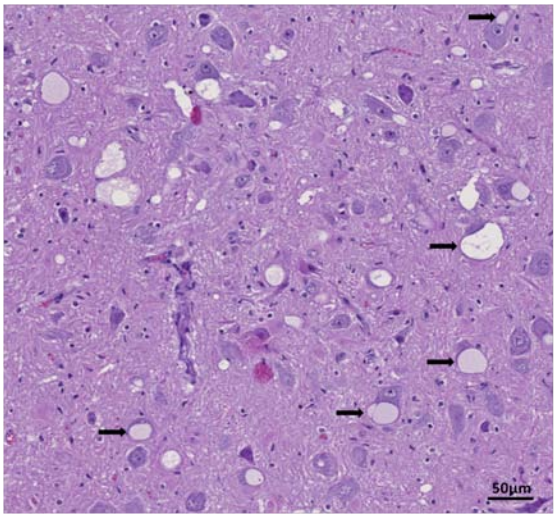

Figure 1 - Mesencephalon. Intraneuronal vacuolisation of the red nucleus. Prominent vacuoles of different sizes are located in the perikaryon of some neuronal bodies (arrows) occupying most of the perikaryon and displacing the neuronal nucleus to the periphery. Small vacuoles are also present in the neuropil together with a moderate gliosis (microgliosis).

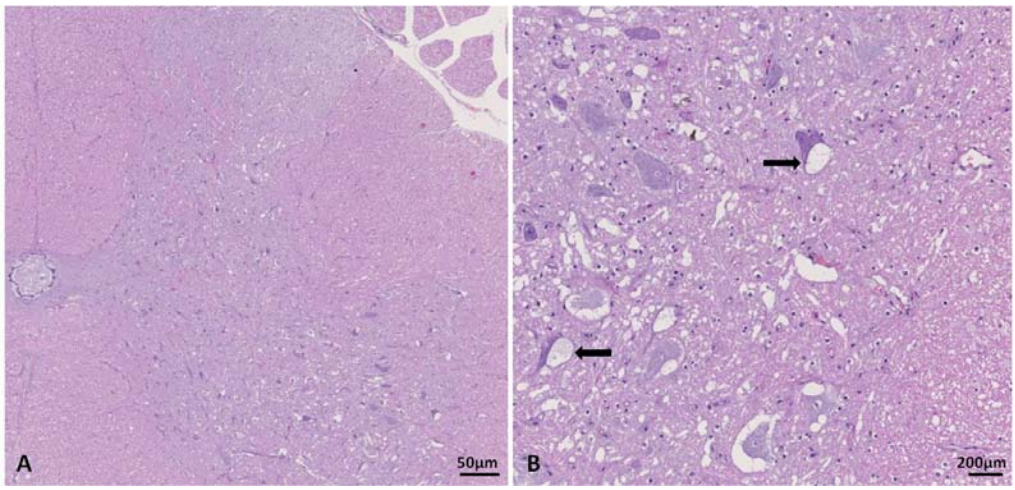

Figure 2 - Lumbo-sacral spinal cord. **(A)** A general view of the lumbo-sacral spinal cord shows a generalised spongiform appearance of the grey matter in the dorsal and ventral spinal horns. **(B)** Detail of the ventral spinal horn showing a moderate spongiosis of the neuropil and the presence of vacuoles of different sizes with an irregular greyish content in most of the perikaryon of the neuronal bodies (arrows). 268x119mm (150 x 150 DPI)

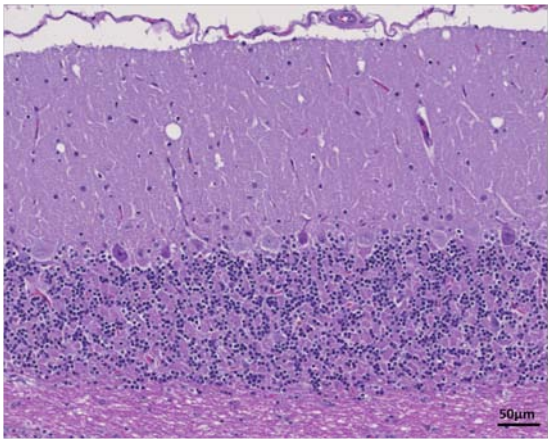

Figure 3 - Cerebellar cortex. High-power-field view of the cerebellar cortex showing a generalised mild spongiosis of the molecular layer with round, empty spaces in the neuropil.

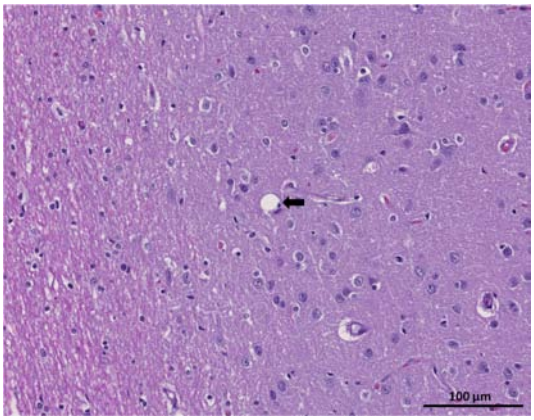

Figure 4 - Cerebral cortex. Presence of prominent and empty vacuoles multifocally distributed through the neuropil of the parietal cortex (arrow). The lesion is located in the deeper layer of parietal cortex while no histopathological changes are observed in the subcortical white matter (left side).

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CAPÍTULO 3

Pain-related aggression in dogs: 12 clinical cases

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ABSTRACT

The aim of this retrospective study was to describe the main features of pain-related aggression in dogs. Twelve dogs presented for aggressive problems at the Veterinary Hospital of the Autonomous University of Barcelona, Spain, were included and a questionnaire was used to gather information on the context of the aggression, body posture during the attack, impulsiveness and aggressive behavior before the onset of the pain-eliciting condition. The most common cause of pain was hip dysplasia (66.7 %), but no relationship was found between the cause of pain and the characteristics of the aggressive behavior. Dogs were classified as having been aggressive before or after the onset of painful condition. Dogs that had not been aggressive before the onset of the pain-eliciting condition were more impulsive (DF=1, chi-square= 5.3, p=0.0209), showed aggression as a result of manipulation context more frequently (DF=1, chi-square= 6, p=0.0143) and adopted a defensive body posture more frequently (DF=1, chi-square= 3.733, p=0.0533) than dogs that had been aggressive before the onset of pain. These results suggest that previous expression of aggressive behavior has a major effect on the pattern of pain-related aggression in dogs.

1. INTRODUCTION

The International Association for the Study of Pain – IASP (IASP, 2011) defines pain as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage.

The most common sign of pain in animals is a change in behavior (Hellyer et al., 2007). Behavioral signs of pain include both the loss of normal behaviors and the development of new and abnormal behaviors. The most common behaviors classified as "lost normal behaviors" are decreased ambulation or activity, lethargic attitude and decreased appetite, and the most common "developed abnormal behaviors" are aggression, fear reactions, inappropriate elimination, vocalization, decreased interaction with other pets or family members, altered facial expression, altered posture, restlessness and hiding (Hellyer et al., 2007).

It is widely accepted that pain can cause aggressive behavior that is often described as a defensive reaction to avoid physical contact that may cause further injury (Rutherford, 2002).

Pain elicits a stress response (Mellor et al., 2000). Pain-induced stress responses lead to increased cortisol, catecholamines and inflammatory mediator that cause tachycardia, vasoconstriction, decreased gastrointestinal motility, delayed healing and sleep deprivation. Pain, specially chronic pain, also causes unseen changes in the central nervous system (CNS) that can lead to magnification of pain perception, through allodynia and hyperalgesia, and a prolonged pain state (Hellyer et al., 2007).

That pain-induced stress response may reduce serotonin activity in the brain (Mellor et al., 2000). Pain could also decrease physical activity and this may further reduce serotonin activity in the CNS (Chaouloff, 1997). A reduction of serotonin activity in the CNS has been related to aggressive behavior in dogs (Tsatsoulis et al., 2006). Whether changes in serotonin activity caused by pain may result in a pattern of aggressive behavior different from that caused by a defensive reaction is unknown.

The aim of this study was to describe the main features of pain-related aggression in dogs, including its context, the dog's body posture and whether the dog reacted impulsively.

2. MATERIAL AND METHODS

Twelve dogs, 11 males (11/12) and 1 female (1/12), presented at the behavioral service of the Veterinary Hospital of the Autonomous University of Barcelona (AUB-VH), Spain,

between 2000 and 2010, were included. None of the dogs was neutered and all of them were purebred dogs. The mean age was 5.08 years old (range 1 - 13 years). All the dogs were diagnosed as having pain-related aggression. A physical and neurological examination was performed on all dogs. Ancillary diagnostic techniques were used as needed. The onset of pain was considered as the onset of clinical signs (behavioral, orthopaedical or other signs of pain). The development of a new aggressive behavior as well as the worsening of previous one, were considered as behavioral signs.

All the diagnoses were based on owners' interviews and medical and imaging tests. The owners of the dogs were interviewed by a trained behaviorist using a questionnaire to gather information on the target of aggression (familiar people, unfamiliar people or other dogs), the contexts in which aggression appeared, the body posture adopted by the dogs during the attacks, the presence of impulsiveness and whether the dog had showed aggressive behavior before the onset of the pain-eliciting condition (table 1). Impulsiveness was considered as a reduction or complete lack of warning signals previous to an attack (Peremans et al., 2003).

Three different contexts were considered: *contact* (aggression occurred when someone tried to touch the dog), *territorial* (aggression occurred when someone came into the dog territory) and *competitive* (aggression occurred when someone challenged the dog over a resource such as food or toys) (Beaver, 2009).

Three different body postures were considered: *offensive* (raised tail, pricked up ears, eyes fixed to the objective and straight forelegs during the attacks), *defensive* (fallen ears, the tail between legs, folded forelegs and averted sight) and *ambivalent* (mixture of offensive and defensive elements) (Overall, 1997; Fatjó, 2007).

A χ^2 analysis (SAS® 9.1 version; software SAS Institute Inc., Cary, NC; 1991-2001) was used to assess the possible associations between variables. A value of $p<0.05$ was considered significant for all analysis.

3. RESULTS

The causes of pain were hip dysplasia (8/12), chronic otitis (2/12), skin injury (1/12) and lameness due to an elbow osteoarthritis (1/12). Five dogs (5/12) had received some sort of treatment to reduce pain and no other treatment (in 4 cases with non-steroidal anti-inflammatory drugs and in one case with a surgical procedure with intra and postsurgical analgesia). Six dogs (6/12) were treated with both NSAIDs and behavioral modification (BM). The remaining dog (1/12) was euthanatized just a few days after the visit. In four of

the five cases treated only for pain (4/5) the follow-up was positive, whereas it was unknown in the other dog (1/5). One of the dogs that were treated with both analgesia and BM (1/6) improved, and in the remaining 5 dogs (5/6) the evolution was unknown. Considering a positive follow-up as a lack or a reduction in frequency and intensity of both medical and behavioral signs of pain, evaluated by a new owners' interview.

In 6 cases (6/12) the dogs were aggressive before the onset of pain (*Aggressive Before the Onset of Pain dogs – ABOP dogs*); the remaining 50% (6/12) had not been aggressive before in the onset of the pain-elicitin condition (*non Aggressive before the Onset of Pain dogs – non-ABOP dogs*). Five *ABOP dogs* (5/6) had been aggressive in competitive contexts and one (1/6) in a territorial context (Table 2).

Non-ABOP dogs showed aggressive behavior in a contact context more frequently than ABOP dogs (DF=1, chi-square= 6, p=0. 0143), whereas *ABOP dogs* showed an increase in the frequency and intensity of the attacks when pain started and the attacks occurred in competitive or territorial contexts after the pain started.

Five *non-ABOP dogs* (5/6) and one *ABOP dog* (1/6) were impulsive (DF=1, chi-square= 5.3, p=0.0209).

Four *non-ABOP dogs* (4/6) showed a defensive body posture and in the other two *non-ABOP* dogs (2/6) the posture was unknown. In *ABOP dogs*, one animal (1/6) showed a defensive body posture, two (2/6) were ambivalent and three (3/6) had an unknown posture. Therefore, *non-ABOP dogs* showed a defensive body posture more frequently than *ABOP dogs* (DF=1, chi-square= 3.733, p=0. 0533).

4. DISCUSSION

There are few epidemiological data available about the incidence of pain-related aggression and its causes. Pain-related aggression is the primary problem in approximately 2% to 3% of referred aggression cases (Beaver, 1983; Borchelt, 1983). In our retrospective study, osteoarthritis (OA) and specifically OA due to hip dysplasia (HD) was the most common cause of pain (8/12). HD is considered to be the most common orthopaedic disease in dogs (Denis, 2009; Kronveit et al., 2010). Reported breed prevalence varies from 2 % to 67 % (Kronveit et al., 2010). In two recent reports HD was present in more than 40 % of Golden Retrievers, Labrador Retrievers and Rottweillers (Paster et al., 2005; Smith et al., 2006). Although HD is a disease affecting most if not all dog breeds, the incidence is lower in small breeds (Priester et al., 1972; Corley et al., 1985). The vast majority of dogs affected with HD show minimal or no clinical signs (Barr et al.,

1987; Ginja et al., 2008). Some chronic hip alterations (i.e. bony remodeling, fibrosis and thickening of the joint capsule) can actually improve joint congruity and stability, which can result in spontaneous improvement in hip-limb function (Ginja et al., 2010). As a result, periods with little or no pain may lead to difficulties in the diagnosis and the possibility of the condition being under-diagnosed. Hence, we could suggest that part of the dogs with hip dysplasia, that remain undiagnosed, could express only behavioral signs, as a worsening of a previous aggressive problem or a development of a new aggressive behavior. Therefore our results suggest that HD may be an important risk factor for aggression in dogs, particularly large ones.

According to our results there are two different patterns of expression of pain-related aggression in dogs depending on whether the dog has shown aggressive behavior before the onset of pain.

A major difference between *non-ABOP* and *ABOP dogs* is that the former showed aggressive behavior in a contact context more frequently than the latter. This may be due to the fact that owners of ABOP dogs are more cautious and avoid touching the dog while it is in pain. Therefore, *ABOP dogs* end up showing aggressive behavior mainly in the same contexts as before the onset of pain but more frequently and with more intensity. The increase in frequency and intensity could be due to a decreased threshold of aggressiveness as a result of the stress response caused by pain. In contrast, owners of *non-ABOP dogs* are less cautious and may elicit a defensive reaction when touching the dog.

Impulsiveness may be related to the owners failing to identify warning signals (Tami et al., 2009), a decreased serotonin activity in the CNS (Reisner, 1996; Peremans, 2003), morphological traits (Fox, 1971) and learning (Pageat, 1998). In addition, some authors suggest that dogs can be impulsive (Bowen et al., 2005) in order to avoid further injury when they are in pain. We suggest that the fact than *non-ABOP* dogs are more impulsive than *ABOP* dogs could be due to two different reasons. First, due to their previous experience with aggressive interactions, *ABOP* dogs may have learnt to modulate their aggression, and we could suggest that dogs would learn to reduce aggressive behavior to the minimum intensity required to achieve the desired goal, to avoid further injury. Second, impulsiveness in *non-ABOP* dogs could simply be the result that non-ABOP dogs are aggressive in a contact context more frequently than *ABOP* dogs and this context per se may elicit an impulsive reaction to avoid further injury.

Our results suggest that previous expression of aggressive behavior has a major effect on the pattern of pain-related aggression in dogs. Finally our results stress the importance of considering and checking medical problems, particularly painful ones, when dealing with aggressive dogs.

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CAPÍTULO 4

Can behavioral problems lead to changes in the hematological and biochemical state of dogs?

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ABSTRACT

Objective

We aim first, to see whether behavioral problems can lead to changes in routinely checked, biochemical and hematological parameters of dogs. Finally, to determine if these changes are clinically relevant, in other words, whether these changes could lead to medical problems in dogs.

Methods

One hundred and twenty-three animals (n=123) were included in the study. Seventy-one of the 123 animals (71/123) had behavioral problems and 52 (52/123) were dogs without any kind of behavioral problem. Nineteen dogs (n=19) showed fear and phobias (without including fear-related aggression), ten (n=10) separation anxiety problems, and fifty-three (n=53) aggressive-related problems (including fear-related aggression). A complete blood count and complete biochemistry profile were performed.

Results

Regarding fear and phobias, statistical differences were found in the WBC, Mono and PMNN values. All three values were statistically higher in the fear/phobias group (n=19) than in the rest of our population (n=103).

Concerning separation anxiety problems, statistical differences were found in the MCV, MCH, Crea and ALT. The MCV, HCM and Crea values were statistically lower in the separation anxiety group (n=10) than in the rest of our population (n=112), whereas the ALT concentration was statistically higher in the separation anxiety group.

Finally, regarding aggressive problems, statistical differences were found in the following parameters: RBC, MCV, MCHC, Lym, N:L ratio, Chol, TP, ALT and Na. The values of RBC, MCHC, N:L ratio, Chol and TP were statistically higher in the aggressive-dogs group (n=53) than in the rest of the population (n=69), whereas the values of MCV, Lym, ALT and Na were lower in the aggressive group than in the rest of the dogs.

Conclusions

Behavioral problems could lead to changes in physiological parameters in dogs. Our results suggest that aggression-related behaviors, separation anxiety and fear-related problems could lead to different consequences in dog physiology.

1. INTRODUCTION

Behavioral problems are common[1]. Among all behavioral problems, the most common are aggression-related problems, separation-related problems and fear-related problems [1,2]. These three behavioral problems could be due to many causes. Nevertheless, the majority of their etiologies may lead to a stress response. Consequently, behavioral problems could be considered as a source of stress. Furthermore, this relationship is bidirectional.

Stress is defined as a complex multidimensional phenomenon promoted by several noxious or unpredictable stimuli (stressors) that cause a physiological response aimed at maintaining or recovering the body´s homeostasis[3]. Stress could be well described in terms of the duration of its effects as acute (transitory) or chronic (long-term) stress. Second, not all types of stress are harmful or negative; in fact, stress could have positive (eustress), negative (distress) or neutral effects[4] and the key to differentiate distress from a non-threatening stress is the biological cost[5]. In general terms, although distress can result from acute stress[5], this is perceived as useful or beneficial and not negative; this is because animals usually have sufficient reserves of biological resources to cope with acute stressors. Then, the biological cost for the animal is minimal. On the other hand, it is easier that these biological reserves will be insufficient if stress response becomes chronic. This loss of reservoirs could result in malfunctions of organs and systems[5]. Most behavioral problems are already chronic when the owner decides to ask a behaviorist for help. Furthermore, stress response can also be affected by the nature of the stressor, its intensity and its predictability [6-8].

Both acute and chronic stress can lead to changes in hematological and biochemical parameters. To study these hematological changes is important for two main reasons: First, because these changes could be useful as stress indicators. Although these changes are not always signs of illness, variations within the physiological values of these parameters could be used as stress indicators. Second, because their study could help to understand the underlying mechanisms of behavioral problems and their consequences on health.

Hematological and biochemical biomarkers have been studied in many different animal species, and in human beings also, in relation to different stressors. Changes in peripheral cell blood count (CBC), hemoglobin concentration, hematocrit, white blood cell count, thyroid hormones, liver enzymes, different ions, proteins, glucose, fructosamine, and urea, among many others, have been documented as a result of stress response [4,9-16].

Specifically, acute stress has been associated with changes in glucose concentration[17], reduction of some plasma minerals (potassium [4,18] and magnesium[19]), increases in urea (usually as an indicator of food deprivation), hematocrit, total protein, albumin, in creatine kinase, LDH and ALT[20,21]. Nevertheless, these changes are usually transitory and for a short period of time (minutes after the stressor). In some cases, the adaptive homeostatic responses observed during acute stress can improve welfare[4].

Other physiological changes have been demonstrated due to chronic stress response. The increase of ALKP concentration [14,22,23], decrease of K concentration[18], changes (increases and decreases depending on the study) of proteins [4,24], and increase of the N:L ratio would be among those most outstanding [25,26].

Plasma cortisol concentration is one of the most studied indicators in both acute and chronic stress. However, it has different limitations: diurnal rhythm [27-30], feeding schedule[31], sex [32-33], reproductive state[32], species and strain[34], individual differences (e.g., due to early life experience)[35], duration of the stressor, and the process of catching and handling the animal[36] are among many factors that could modify plasma glucocorticoid concentration and/or its interpretation as a stress indicator. Furthermore, plasma cortisol concentration is not routinely checked in a general approach of a dog's health status. Thus, it could be interesting to study other possible hematological parameters, checked routinely if possible, which may help to identify the stress response and/or its consequences on health.

The effect of dogs' behavioral problems, as a source of stress, on hematological and biochemical parameters is poorly understood. The majority of these studies in dogs have focused on the lipid and thyroid panel and its relation to aggressive behaviors. Low concentrations of total cholesterol, triglycerides, and high-density lipoprotein-cholesterol (HDL) have been found in aggressive dogs, as compared to non-aggressive dogs[37]. It is important to highlight that these studies have been performed in specific diagnoses of aggression (specifically, dominance aggression and learned aggression). However, the effect of other sorts of aggressive behaviors, even of dominance and learned aggression, remains unclear. On the other hand, in clinical reports, hypothyroidism has been classically associated with aggression, and also with apathy, lethargy or mental dullness, exercise intolerance and decreased libido [38,39]. The direction of the relationship between aggressive behaviors and hypothyroidism remains unclear, if, in fact, it really exists. And it remains unclear, first, because this could be just a co-morbid relationship[40] that co-exists in time. Second, because it is well established that exogenous glucocorticoids can lead to

changes in thyroid hormone concentration[41]. Then, it may be hypothesized that endogenous glucocorticoids could also modify thyroid hormone concentration. Nevertheless, different studies have failed in trying to demonstrate this hypothesis in aggressive dogs[11].

Finally, different CBC parameters have also been used as stress indicators, especially those that involve leukocytes. The neutrophils/lymphocytes ratio has been proposed as a stress indicator both in veterinary and human medicine. Furthermore, it is considered an indicator of poor prognosis in many cancers and vascular diseases [42-46]. It may be an also inexpensive and useful biomarker of stress in dogs. However, evidence concerning the effect of behavioral problems in dogs using this ratio is very poor[47-48].

Thus, in this study we aim first, to see whether behavioral problems can lead to changes in routinely checked, biochemical and hematological parameters of dogs. Finally, to determine if these changes are clinically relevant, in other words, whether these changes could lead to medical problems in dogs.

2. MATERIAL AND METHODS

Animals

One hundred and twenty-three animals (n=123) were included in the study. All of them were patients of the behavioral medicine service of the Fundació Hospital Clínic Veterinari at the Veterinary School of the Autonomous University of Barcelona (UAB), a referral veterinary center. All dogs were visited by a veterinary behaviorist (diplomate from the European College Animal Welfare and Behavioral Medicine).

Sixty-seven dogs were male (67/123) and 56 were female (56/123). Fifty-one were neutered (51/123) and 72 were entire (72/123). Twenty-seven were castrated males (27/67) and 24 spayed females (24/56). Thirteen dogs (13/123) were younger than one year old, 100 dogs (100/123) were from 1 year old to 7 years old, and ten dogs (10/123) were older than 7 years old.

Behavioral problems

Behavioral problems included in the study were separation anxiety, competitive aggression toward familiar people, competitive aggression toward familiar dogs, possessive aggression toward familiar people, fear-related aggression toward people, fear-related aggression toward dogs, territorial aggression, intra-sexual aggression, fear or

phobia of noise, fear or phobia of other stimuli, generalized fear or phobia, compulsive disorders and others.

Blood parameters

A complete blood count (CBC) and a complete biochemistry profile were performed for each sample.

CBC included red blood cell count (RBC), hematocrit (HCT), mean corpuscular volume (MCV), hemoglobin (HGB), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell count (WBC), band neutrophils (BNEU), polymorphonuclear neutrophils (PMN), lymphocytes (Lym), monocytes (Mono), eosinophils (Eos), basophils (Baso) and platelet count (PLT).

The biochemistry profile included bile acids (BileA), alanine transaminase or alanine aminotransferase (ALT), aspartate transaminase or aspartate aminotransferase (AST), alkaline phosphatase (ALKP), gamma-glutamyl transpeptidase (GGT), total bilirubin (TBil), cholesterol (Chol), triglycerides (Tryg), total calcium (Ca), chlorine (Cl), phosphorous (P), sodium (Na), potassium (K), creatinine (Crea), blood urea nitrogen (BUN), creatine kinase (CK), glucose (Glu), fructosamine (Fruc), total T4 (tT4) and thyrotropin or thyroid-stimulation hormone (TSH).

A total protein test was performed, including total protein (TP), total albumin (ALB), total globulins (TGlob), α1- globulins (α1-Glob), α2- globulins (α2-Glob), β-globulins (β-Glob) and γ-globulins (γ-Glob).

Methodology

Dogs with behavioral problems

All of these dogs were referred to the behavioral medicine service due to a behavioral problem. Each behavioral consultation consisted of an interview with the owners, a complete physical and neurological examination and blood collection. All blood samples were collected, after at least an 8-hour fast, through venipuncture of the jugular vein. All samples were processed within the first 12h after the extraction. None of the animals had ever received antidepressant drugs.

Dogs without behavioral problems

All dogs without behavioral problems were recruited through advertising in the Veterinary School of the UAB, in the Fundació Hospital Clinic Veterinary of the UAB, and in

the Research Institute in Semiochemistry and Applied Ethology (IRSEA – Quartier Salignan, Apt, 84 400, France). All of them underwent exactly the same procedures as dogs with behavioral problems. None of the animals had ever received antidepressant drugs

Tests

All biochemistry panels were analyzed in the Servei de bioquímica clínica - Veterinary School of the UAB (08193, Bellaterra, Spain), and all CBC were analyzed in the Servei d'hematologia Clinica Veterinària (SHCV) - Veterinary School of the UAB (08193, Bellaterra, Spain).

3. RESULTS

Seventy-one of the 123 animals (71/123) had behavioral problems and 52 (52/123) were dogs without any kind of behavioral problem.

Of the 71 dogs with behavioral problems, 69 animals (69/71) showed stress-related problems, while two (2/71) showed only behavioral problems that were not related to stress response (one showed pseudopregnancy and the other had an elimination problem due to incomplete house-training). Of the sixty-nine animals with stress-related behavioral problems, thirty-nine showed one behavioral problem (39/69), while thirty had more than one behavioral problem (30/69).

Of the 123 animals, ten showed separation anxiety (10/123). Nineteen dogs showed competitive aggression toward familiar people (19/123), and eight dogs showed competitive aggression toward familiar dogs (8/123). Possessive aggression toward familiar people was diagnosed in four dogs (4/123). Fear-related aggression toward people was diagnosed in twenty-three dogs (23/123), and nine dogs showed fear-related aggression toward other dogs (9/123). Territorial aggression was diagnosed in four dogs (4/123), and intra-sexual aggression in six dogs (6/123). Fear or phobia of noise was diagnosed in fifteen cases (15/123), fear or phobia of other stimuli in six dogs (6/123), generalized fear or phobia in one case (1/123), and compulsive disorders were seen in five dogs (5/123).

For the statistical analyses, the problems were grouped into three major categories: Fear and phobias (without including fear-related aggression)(n=19), separation anxiety problems (n=10) and aggressive-related problems (including fear-related aggression)(n=53). Compulsive disorders (n=5) and others (n=2) were excluded from the study due to the low number of cases. It is important to highlight that there were dogs (n=30) with more than one behavioral problem.

Regarding fear and phobias (Table 1), statistical differences were found in the WBC, Mono and PMNN values. All three values were statistically higher in the fear/phobias group (n=19) than in the rest of our population (n=103).

Concerning separation anxiety problems (Table 2), statistical differences were found in the MCV, MCH, Crea and ALT. The MCV, HCM and Crea values were statistically lower in the separation anxiety group (n=10) than in the rest of our population (n=112), whereas the ALT concentration was statistically higher in the separation anxiety group.

Finally, regarding aggressive problems (Table 3), statistical differences were found in the following parameters: RBC, MCV, MCHC, Lym, N:L ratio (Table 4 and Figure 1), Chol, TP, ALT and Na. The values of RBC, MCHC, N:L ratio, Chol and TP were statistically higher in the aggressive-dogs group (n=53) than in the rest of the population (n=69), whereas the values of MCV, Lym, ALT and Na were lower in the aggressive group than in the rest of the dogs.

All the parameters not mentioned above remained without statistical differences between the groups.

Table 1 - Comparison of fear/phobia group (n=19) against the rest of the dogs (n=103). Parameters statistically different. Only statistically different parameters have been included ($p<0.05$).

	Fear/phobia Mean ± SD (range)	Non-fear/phobia Mean ± SD (range)	Reference range	p-value
WBC (x/µL)	10138.95 ± 2670.72	9244.84 ± 2485.15	6000 – 17000	0.0274
Mono (x/µL)	577.47 ± 478.65	415.60 ± 250.98	150 – 1350	0.0126
PMNN (x/µL)	6282.63 ± 1573.50	5596.94 ± 1701.66	3000 - 11500	0.0156

Table 2 - Comparison of separation anxiety group (n=10) against the rest of the dogs (n=112). Parameters statistically different. Only statistically different parameters have been included ($p<0.05$).

	Separation anxiety Mean ± SD (range)	Non-separation anxiety Mean ± SD (range)	Reference range	p-value
MCV (fl)	67.00 ± 4.03	71.57 ± 4.73	62 – 77	0.0083
MCH (pg)	22.21 ± 1.45	23.49 ± 1.32	21.5 – 26.5	0.0066
Crea (mg/dL)	0.97 ± 0.15	1.07 ± 0.16	0.5 – 1.5	0.0304
ALT (UI/L)	72.60 ± 80.55	49.32 ± 50.80	21 – 102	0.0109

Table 3 - Comparison of aggressive-dogs group (n=53) against the rest of the dogs (n=69). Parameters statistically different. Only statistically different parameters have been included ($p<0.05$).

	Aggressive problems Mean ± SD (range)	Non-aggressive Mean ± SD (range)	Reference range	p-value
RBC (x106/µL)	7.20 ± 0.80	6.80 ± 0.70	5.5 – 8.5	0.0059
Lym (x/µL)	2450.00 ± 1177.00	2792.36 ± 1105.11	1000 – 4800	0.0175
MCHC (g/dL)	33.86 ± 1.33	33.15 ± 1.42	33 – 37	0.0193
MCV (fl)	69.50 ± 4.40	72.40 ± 4.70	62 – 77	0.0038
ALT (UI/L)	43.43 ± 68.39	57.21 ± 22.39	21 – 102	0.0050
Chol (mg/dL)	236.40 ± 53.95	222.20 ± 56.06	135 – 270	0.0360
TP (g/dL)	6.43 ± 0.51	6.26 ± 0.46	5.4 – 7.1	0.0124
Na (mmol/L)	146.99 ± 2.33	148.50 ± 3.43	141 – 152	0.0171

Table 4 - Neutrophil/lymphocyte ratio (Mean ± SD). Only statistically different parameters have been included (p<0.05).			
	Problem	Non-problem	p-value
Aggressive	3.1 ± 2.09	2.3 ± 1.02	0.0079
Fear	2.7 ± 1.11	2.7 ± 1.72	0.5394
Separation Anxiety	2.3 ± 0.72	2.7 ± 1.67	0.5998

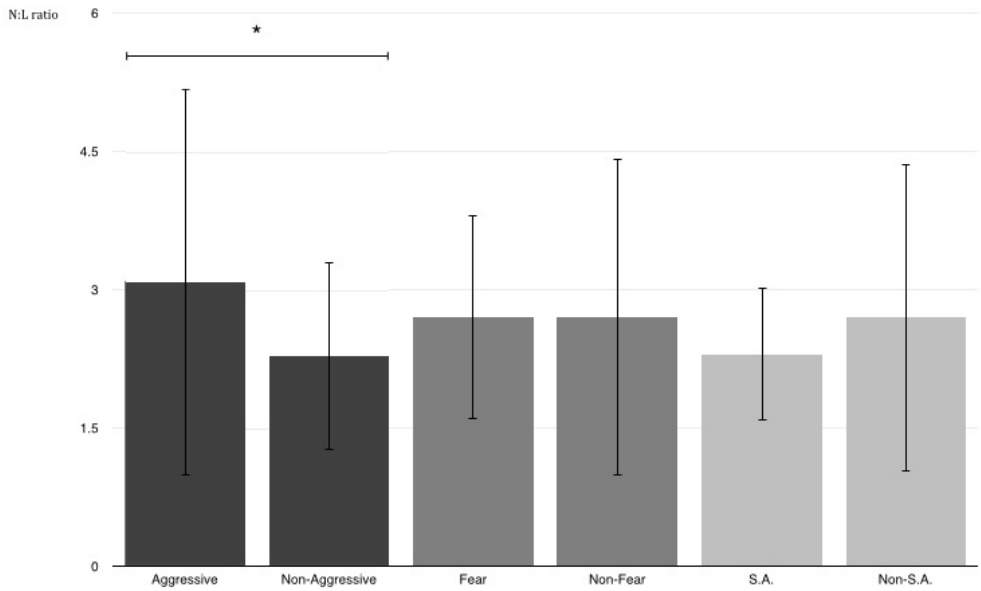

Figura 1 - Neutrophil/lymphocyte ratio (Mean ± SD) for each group. S.A.: Separation Anxiety. N:L ratio: neutrophils/lymphocytes ratio. (P-value <0.01).

4. STATISTICAL ANALYSIS

Data from the complete blood count and biochemistry profile (except ALT and BileA) were analyzed using the GLM procedure of SAS (SAS Institute Inc.; Cary, NC). A log transformation was applied to those parameters whose residuals were not normally distributed (8 parameters: PMN, Eos, AST, CK, ALKP, Tryg, tT4, TSH, neutrophils/lymphocytes ratio). ALT and BileA were analyzed by means of a GENMOD procedure for repeated measures. A negative binomial distribution was used according to the value of the deviance. The model included the effect of the presence of aggressive behavior (2 levels), separation anxiety (2 levels) and fear and phobia (2 levels) as well as the effect of gender, reproductive state and age. The residual maximum likelihood was used as a method of estimation and the least square means of fixed effects (LSMEANS) was used when analysis of variance indicated differences ($P < 0.05$).

5. DISCUSSION

This study has found four important results: (1) Dogs with separation anxiety showed increased values of ALT. (2) Aggressive dogs had increased values of total cholesterol. (3) No differences were found regarding thyroid hormones in any of the behavioral problems. (4) The neutrophil/lymphocytes ratio was higher in aggressive dogs.

Dogs with separation anxiety showed increased values of ALT (1). Increased concentration of ALT is principally caused by hepatocellular damage in dogs[49]. The mean of our population was within the physiological limits (mean 72.6 UI/L ± 80.55 UI/L. Reference value: 21-102 UI/L). Nevertheless, taking into account the standard deviation, there are animals that could move outside the reference values. We suggest that these changes are due to the behavioral problems because none of the dogs had received any treatment at the moment of sampling. Then, we suggest that ALT concentration should be monitored in dogs with separation anxiety, especially in those that have to receive drugs with hepatic metabolism as a part of their treatment (the majority). We are completely aware about the low number of animals with separation anxiety in the present study (n=10). The underlying mechanism of that relation remains unclear, however, this result could be a good starting-point for further investigation with a higher number of cases in a case-control study.

Although the mean was always within the normal limits, in contrast to previous studies, (2) aggressive dogs showed a higher concentration of total cholesterol (Chol). One previous study demonstrated low concentrations of total cholesterol, low concentrations of serum triglyceride, and low concentrations of high-density lipoprotein-cholesterol (HDL) in dominance-aggressive dogs, as compared to non-aggressive dogs[37]. Another study (performed with military patrol dogs trained for controlled aggression) found a negative correlation between the degree of aggressiveness with total cholesterol and HDL[50], and failed to find differences with low-density lipoprotein-cholesterol (LDL), very low-density lipoprotein-cholesterol (VLDL), and triglycerides, among other parameters.

There is one main difference between our study and the others [37,50]. We evaluated the expression of a certain behavior (aggressive behavior), more than a specific diagnosis. One possible explanation for these contradictory results would be that the different diagnosis for aggressive behavior (i.e., territorial aggression, fear-related aggression, etc.) could have a different effect on cholesterol concentration. Then, our results would represent the "final result", in terms of cholesterol concentration, of being aggressive. Each one of the other studies was based on one sort of aggressive behavior. The first was based on

dominance aggression[37], and the second on learned aggression[50]. Today, dominance aggression as a diagnosis has been, at least, questioned. This means that it is likely that more than one kind of diagnoses were wrongly included in this study[37], and this makes it difficult to compare it with our results. Further investigation is needed in order to elucidate the true role of aggressiveness on cholesterol concentration (and other lipid parameters) and vice versa.

On the other hand, some authors have linked hypothyroidism to aggressive behavior[38]. Hypercholesterolemia has commonly been described in hypothyroid dogs[41]. However, in our study, in accordance to previous ones [11,51], (3) no differences were found between aggressive and non-aggressive dogs in total T4 and TSH. As we have indicated in the Introduction, it may be hypothesized that endogenous glucocorticoids could modify thyroid hormone concentration. Taking into account the present study and previous ones, it seems that thyroid hormones would not be modified by behavioral problems in dogs. Nevertheless, some case-report articles have demonstrated a relation between aggressive behaviors and hypothyroidism. Now this relationship could be explained: first, as a co-morbid situation that co-exists in time without a true causative relationship and, second, because the direction of the relation is from hypothyroidism to aggressive behaviors. In fact, some new neuro-imaging research suggests that brain structure and function are altered in hypothyroid patients, with decreased hippocampal volume, cerebral blood flow and function globally, and in regions that mediate attention, working memory and motor speed in human beings and in rats [52-56]. Additionally, a recent study[55] showed that some of these alterations (alterations in working memory and abnormalities in functional magnetic resonance) were no longer present after six months of treatment with levothyroxine.

Finally, the present study shows that the (4) N:L ratio increased in aggressive dogs. To the best of our knowledge, this is the first study that correlates this increase with aggressive behaviors in dogs. It is well-known that stress can lead to changes in WBC and immunity, both in veterinary and human medicine. This could be a long-term effect. As an example, one study[57] in human medicine found differences in WBC, among other immunological parameters, 20 years after exposure to severe stress (the study was carried out with Vietnam veterans). N:L is considered a good indicator of stress in human medicine as well as in veterinary medicine[25]. For example, different studies found increases of the N:L ratio as a result of transport in cattle, goats, horses and swine[58]. In companion animals, two studies evaluated the effect of transport on dog stress using the N:L ratio[47-48]. Both

studies showed that the N:L ratio is also a good stress-indicator in dogs. Our result shows that this ratio can also change due to another source of stress, that is, aggressive behaviors.

Secondly, we wished to determine whether these changes are clinically relevant. In light of our results, it is difficult to establish a direct relationship between the behavioral problems studied and any medical problem. Nevertheless, the N:L ratio has been found to be a good predictor of cardiovascular risk in humane medicine, better than high neutrophil counts or low lymphocyte counts separately[42]. An elevated N:L ratio has also been used as a predictor of poor prognosis of some types of lung cancer[43], colorectal cancer[44], and cervical cancer[45] among others. The N:L ratio has also been associated with more susceptibility to infections in birds[46]. Thus, for future research, it could be interesting to study whether aggressive behavior may decrease dogs' capability to cope with infectious, cardiovascular diseases, cancer problems or, even, with the aggressive behavior itself.

The limitations of this study include that this is not a case-control study, and the small sample size regarding separation anxiety. Nevertheless, it could be the first step in order to establish what the hematological and biochemical parameters could be in order to study more in-depth in the future, concerning behavioral problems in dogs.

Finally, today it is well-known that, contrary to the initial beliefs, the stress response would be different depending on the stressor and, in fact, it is determined by the nature of this stressor and by the emotional processes that this arouses within each individual[59]. Our results suggest that aggression-related behaviors, separation anxiety and fear-related problems could lead to different consequences in dog physiology.

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CAPÍTULO 5

Effect of fluoxetine hydrochloride on the biochemistry profile and complete blood count in healthy dogs

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ABSTRACT

Objective

The aim of this study is to evaluate the effect of fluoxetine on any haematological and biochemical parameter that could, in turn, affect the behaviour of the dog.

Methods

Thirteen healthy dogs without medical or behavioural problems were included in the study. All of them received 1 mg/kg q24h of fluoxetine for 45 days (induction period), and then 1 mg/Kg q48h for 15 more days (withdrawal period). Blood was collected on Days 0, 46 and 61. A complete blood count and complete biochemistry profile were performed.

Results

Regarding the complete blood count, the most important results that were obtained were related to HCT, MCV, MCHC and Mono. In relation to the biochemistry panel, statistical differences were found in the ALT, AST, Cl, CK, Glu, Fruc and Na values.

Conclusions

Despite these changes, we can conclude that the use of fluoxetine in dogs is safe, and does not seem to change any parameter that could affect the behaviour of the animal.

1. INTRODUCTION

Aggression and anxiety-related disorders account for the vast majority of all behavioural disorders in dogs, and their treatment often includes the use of psychotropic drugs. Selective serotonin reuptake inhibitors (SSRIs) are one of the most commonly used drugs in behavioural medicine, and fluoxetine in particular has become the most commonly used SSRI in dogs.

Fluoxetine is a strong inhibitor of serotonin reuptake and a very weak inhibitor of norepinephrine reuptake. It also has very little binding to muscarinic, histaminergic, and alpha1-adrenergic receptors, as compared with other antidepressants[1]. It is well-absorbed after oral administration, although food may delay its absorption. Fluoxetine is largely metabolized in the liver by the cytochrome P-450 enzyme system to norfluoxetine, an equipotent SSRI that contributes to the efficacy of fluoxetine[2,3]. After a single dose of approximately 2 mg/Kg body-weight, fluoxetine has a T1/2 of 6.2h ± 0.8h (mean ± standard error), whereas that of norfluoxetine is of 49.0h ± 3.0h. In a 21-day study, after an administration of 0.75 mg/kg/24h, 1.25 mg/kg/24h and 3.0 mg/kg/24h of fluoxetine in laboratory beagles, a steady state appeared to be reached within 10 days[4]. In a one-year study, dogs were administered 1 mg/Kg/24h dose of fluoxetine, and a continuous increase in trough concentration (plasma concentration of a drug just before the next dose) was observed throughout the year. A similar increase in concentration was observed with norfluoxetine. This phenomenon was not observed at higher doses[4]. Fluoxetine and norfluoxetine are distributed throughout the body, with higher levels found in the lung and liver. CNS concentrations are detected 1h after dosing[5]. Excretion of fluoxetine is primarily via the kidney. In humans, there is a wide variation in duration of action. Liver, but not renal, impairment will increase clearance time[5].

Fluoxetine has been widely used for treatment of affective aggression (especially impulsive ones) and anxiety-related disorders (e.g. separation anxiety, fears and phobias and compulsive disorders) in dogs[6,7]. Analgesic activity is another desired effect of fluoxetine which results, in part, from an increase in the activity of the endogenous descending analgesic system and the central opioid pathways[8]. Nevertheless, this analgesic activity is controversial since many studies show that fluoxetine could enhance pain response[9]. Fluoxetine could also have anti-inflammatory effects[10].

The most common side-effects of fluoxetine are vomiting, diarrhoea, changes in urine frequency, insomnia, sedation, excitement, seizures, headache, abnormal bleeding,

decreased sexual motivation (although in human beings, delayed orgasm or anorgasmia is more common than is a decrease in sexual motivation), anxiety, tremors and changes in appetite[2]. Although it is controversial, some studies show that fluoxetine may increase suicide thoughts in human patients[11].

There are few studies about the effect of fluoxetine on the biochemistry panel and complete blood count. However, It is well-known that fluoxetine may alter the metabolism of blood glucose. In particular, hyperglycaemia may develop during treatment with fluoxetine, while hypoglycaemia may develop upon withdrawal of fluoxetine[1]. Fluoxetine may increase liver enzymes, although there are no reports of liver pathology unless the patient had prior liver disease[5]. Hyponatraemia has been described in human medicine, particularly in elderly patients[12-15]. The vast majority of these studies were retrospective and they were made in human patients with some kind of psychiatric disorder.

Finally, one prospective study showed a relationship between the use of fluoxetine and changes in thyroid hormones[16], which has an important role in human and animal behaviour. A relationship between lipid profiles and changes in behaviour also has been suggested in both animals and human beings, such as major depression[17], generalized anxiety[18], Asperger Syndrome[19] and obsessive-compulsive disorders[20,21].

To the best of our knowledge there is no previous study on the effects of fluoxetine on the biochemistry profile and a complete blood count in dogs. Therefore, the aim of this study is to quantify such effects in order to assess if fluoxetine is a safe drug in dogs and whether it can modify any haematologic and biochemical parameter that could, in turn, affect the behaviour of the dog.

2. MATERIAL AND METHODS

Animals

Thirteen animals (n=13) were included (Table 1) in the study. All of them were patients of a private clinical service (Clínica Veterinaria Balmes) in Palma de Mallorca, Spain. None of them had had behavioural problems, and they had come for a routine visit (vaccination). One of them (Table 1 – No. 12) had suffered a unilateral Legg-Calvé-Perthes, which was solved with surgical treatment (arthroplasty) at nine months old. Another individual (Table 1 – No. 7) had hip dysplasia diagnosed three years before the study, but no treatment was required at the moment of the study.

Eight dogs (8/13) were purebreds. Eight (8/13) were males and five (5/13) were females. Two males were castrated (2/8) and three females were spayed (3/5). The mean age was 7.42 years (2 years ≤ x ≥ 13 years).

One week after initiating the study, one dog (Table 1 – No. 4) was bitten, and he required antibiotic and anti-inflammatory treatment. Fluoxetine treatment was stopped and the animal was discarded from the study.

Methodology

From Day 1 to Day 46 all dogs received 1 mg/Kg q24h of fluoxetine (Fluoxetine Cinfa® 20 mg) in the morning and on an empty stomach. From Day 46 to Day 61 they received 1 mg/Kg q48h in the same conditions described above. Blood was collected on Days 0 (t0), 46 (t1) and 61 (t2) through venepuncture of the jugular vein. All samples were processed within the first 48h after extraction in Vetlab-Idexx Laboratories S.L. (c/ Plom n 2 – 8, 3rd 08038, Barcelona, Spain).

A complete blood count (CBC) and a complete biochemistry profile were performed for each sample. CBC included red-blood-cell count (RBC), haematocrit (HCT), red-blood-cell distribution width (RDW), mean corpuscular volume (MCV), haemoglobin (HGB), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white-blood-cell count (WBC), band neutrophils, polymorphonuclear neutrophils (PMN), lymphocytes (Lym), monocytes (Mono), eosinophils (Eos), basophils (Baso), platelet count (PLT) and mean platelet volume (MPV).

The biochemistry profile included bile acids (BileA), alanine transaminase or alanine aminotransferase (ALT), aspartate transaminase or aspartate aminotransferase (AST), total bilirubin (TBil), total calcium (Ca), chlorine (Cl), cholesterol (Chol), creatinine (Crea), creatine kinase (CK), alkaline phosphatase (ALKP), phosphorous (P), gamma-glutamyl transpeptidase (GGT), glucose (Glu), sodium (Na), potassium (K), sodium-potassium ratio (Na/K), triglycerides (Tryg), blood urea nitrogen (BUN), fructosamine (Fruc), total T4 (T4t) and thyrotropin or thyroid-stimulation hormone (TSH). A coagulation profile including prothrombin time (PT), activated partial thromboplastin time (aPTT) and thrombin time (TT) was obtained. Also, a total-protein test was performed including total protein (TP), total albumin (ALB), total globulins (TGlob), α1-globulins (α1-Glob), α2-globulins (α2-Glob), β-globulins (β-Glob) and γ-globulins (γ-Glob).

3. STATISTICAL ANALYSIS

Data from the complete blood count and biochemistry profile (except ALT and BileA) were analysed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC) for repeated measures. A log transformation was applied to those parameters whose residuals were not normaly distributed (eight parameters: PMNN, Eos, AST, CK, ALKP, Trig, T4, TSH). ALT and BilA were analysed by means of a GENMOD procedure for repeated measures. A negative-binomial distribution was used according to the value of the deviance. The models studied the evolution of each biological variable over treatment and accounted for the effect of the gender as well as the interaction time of extraction by gender. The residual maximum likelihood was used as a method of estimation and the least square means of fixed effects (LSMEANS) was used when analysis of variance indicated differences ($P < 0.05$).

4. RESULTS

Regarding the complete blood count (Table 2), the most important results that we obtained were related to HCT, MCV, MCHC and Mono. HCT remained constant after the first 45 days (t0 – t1) of treatment (induction period), and then it increased during the withdrawal period (t1 – t2). No differences were found between t0 and t2. MCV increased during the withdrawal period, whereas it showed no statistically significant changes during the induction period. In contrast, MCHC decreased during the withdrawal period and remained constant during the induction period. Mono decreased in the withdrawal period, but no differences were found between t0 and t1, nor between t0 and t2. In spite of these variations, all of these parameters were always within the physiological range.

In relation to the biochemistry panel (Table 3), statistical differences were found in the ALT, AST, Cl, CK, Glu, Fruc and Na values (See Table 3).

Regarding the hepatic values, no statistical differences were found in ALT values between t0-t1 and t1-t2, but ALT decreased between t0-t2. AST values decreased in the induction period, but no differences were found between t1-t2 and t0-t2. GGT decreased during the induction period and recovered its initial values during the withdrawal period.

In relation to the ion panel, Cl decreased in the induction period, and it remained low during the withdrawal period. In contrast, Na increased during t0-t1 and remained high between t1-t2.

Concerning the glucaemic panel, the differences between Gluc and Fruc are noteworthy. Glu decreased during the induction period and recovered its initial value after the

withdrawal period. In contrast, Fruc increased during the induction period and recovered its initial value in t2.

CK increased during the induction period and remained high at t2. No differences were found in the thyroid panel.

Regarding the coagulation profile (Table 4), PT was the only parameter which showed a treatment effect: it increased in the induction period and remained high at t2.

In relation to the total-protein test (Table 5), differences were found in α1-glob, α2-glob and TGlob. α1-glob decreased during the induction period and remained low after the withdrawal period, whereas α2-glob increased betweent1-t2 (no differences were found between t0-t1 and t1-t2). Finally there was a sex-treatment interaction in γ-Glob, so that in female dogs it decreased during t0-t1 and remained low in t2.

Table 1 - Animals included in the study

ID	**Name**	**Age (years)**	**Breed**	**Sex**	**Neutered**
1	Avalancha	7	French Bulldog	Female	Y
2	Twister	2,5	French Bulldog	Male	Y
3	Hugo	7	Boxer	Male	N
4	Trui	8	Dalmatian	Male	N
5	Truy	5	Crossbred dog	Male	N
6	León	10	Crossbred dog	Male	N
7	Bubi	13	Golden retriever	Male	Y
8	Estrella	12	Crossbred dog	Female	Y
9	Bruixa	10	Crossbred dog	Female	Y
10	Chuski	8	Crossbred dog	Male	N
11	Bruce	3	English Bulldog	Male	N
12	Xima	9	Ca rater mallorquí	Female	N
13	Clapeta	2	Ca rater mallorquí	Female	N

Table 2 - Complete blood count parameters – Mean ± Standard Deviation			
Parameter (units)	**T0**	**T1**	**T2**
RBC (x1000.000)	7.29 ± 0.74	6.85 ± 0.95	7.00 ± 0.78
HCT (%)	52.18 ± 5.62^{ab}	49.63 ± 6.09^{b}	55.03 ± 5.08^{a}
RDW (%)	16.28 ± 1.83	15.72 ± 1.80	15.50 ± 2.34
MCV (fl)	71.72 ± 5.63^{b}	72.67 ± 3.48^{b}	78.85 ± 3.79^{a}
HGB (g/dl)	17.35 ± 1.81	16.38 ± 2.34	16.78 ± 1.88
MCH (Pg)	23.81 ± 1.23	23.90 ± 1.08	24.00 ± 1.08
MCHC (g/dl)	33.28 ± 1.53^{a}	32.93 ± 1.06^{a}	30.46 ± 1.17^{b}
WBC (x1.000)	8.38 ± 1.54	9.01 ± 2.03	8.65 ± 1.54
BandN (cls/µl)	0 ± 0	0 ± 0	0.5 ± 1,73
PMNN (cls/µl)	5775.33 ± 1405.71	6114.67 ± 1554.75	6216.08 ± 1060.95
Lym (cls/µl)	1758.00 ± 528.60	1942.00 ± 622.62	1654.67 ± 478.05
Mono (cls/µl)	466.92 ± 203.10^{ab}	616.58 ± 311.03^{a}	340.00 ± 162.63^{b}
Eos (cls/µl)	378.25 ± 226.29	330.91 ± 168.14	431.16 ± 294.36
Baso (cls/µl)	6.25 ± 7.89	6.66 ± 8.30	7.64 ± 7.74
PLT (x1000)	295.92 ± 108.87	268.58 ± 76.92	267.25 ± 104.85
MPV (fl)	11.83 ± 1.33	11.75 ± 1.01	11.13 ± 1.73
Different letters within row means significant differences between extractions at $p<0.05$			

Table 3 - Biochemistry profile – Mean ± Standard Deviation			
Parameter (units)	**T0**	**T1**	**T2**
BileA (µmol/l)	3.00 ± 3.31	3.67 ± 3.67	3.00 ± 2.26
ALT (IU/L)	53.00 ± 25.72 [a]	47.40 ± 24.38 [ab]	39.22 ± 19.47 [b]
AST (IU/L)	31.92 ± 15.40 [a]	20.75 ± 9.15 [b]	26.33 ± 11.88 [ab]
TBil (mg/dl)	0.16 ± 0.03	0.21 ± 0.05	0.18 ± 0.04
Ca (md/dl)	10.71 ± 0.34	10.20 ± 0.36	10.48 ± 0.50
Cl (mmol/L)	119.00 ± 4.88 [a]	110.83 ± 2.08 [b]	111.54 ± 2.84 [b]
Chol (mg/dl)	220.50 ± 68.98	239.42 ± 90.58	249.25 ± 95.21
Crea (mg/dl)	0.99 ± 0.20	1.00 ± 0.19	1.01 ± 0.23
CK (IU/L)	35.41 ± 16.52 [b]	80.67 ± 24.34 [a]	73.33 ± 41.05 [a]
ALKP (IU/L)	45.75 ± 15.83	52.73 ± 28.34	48.89 ± 15.00
P (mg/dl)	4.41 ± 0.58	4.22 ± 0.67	4.05 ± 0.60
GGT (IU/L)	5.54 ± 1.97 [a]	4.36 ± 1.86 [b]	5.45 ± 1.50 [a]
Glu (mg/dl)	103.00 ± 10.68 [a]	93.08 ± 4.54 [b]	107.83 ± 16.63 [a]
Na (mEq/L)	143.09 ± 2.43 [b]	146.25 ± 1.91 [a]	146.82 ± 2.09 [a]
K (mEq/L)	4.70 ± 0.41	4.75 ± 0.35	4.89 ± 0.58
Na/K	30.27 ± 2.30	30.92 ± 1.99	29.34 ± 2.13
Trig (mg/dl)	71.75 ± 21.77	102.33 ± 100.29	119.42 ± 130.97
BUN (mg/dl)	27.72 ± 6.54	33.09 ± 9.30	31.25 ± 10.68
Fruc (µmol/l)	218.40 ± 39.59 [c]	274.09 ± 29.92 [a]	245.45 ± 19.53 [b]
T4 (µg/dl)	1.01 ± 0.38	1.16 ± 0.38	1.31 ± 0.42
TSH (ng/ml)	0.20 ± 0.11	0.18 ± 0.10	0.18 ± 0.09
Different letters within row means significant differences between extractions at $p<0.05$			

Table 4 - Coagulation profile – Mean ± Standard Deviation			
Parameter (units)	**T0**	**T1**	**T2**
PT (s)	8.54 ± 0.49 [b]	9.30 ± 0.80 [a]	9.55 ± 0.76 [a]
aPTT (s)	11.34 ± 0.97	11.08 ± 1.04	11.54 ± 0.83
TT (s)	14.48 ± 0.89	13.26 ± 1.29	13.64 ± 1.32
Different letters within row means significant differences between extractions at p<0.05			

Table 5 - Total protein test – Mean ± Standard Deviation			
Parameter (units)	**T0**	**T1**	**T2**
TP (g/L)	67.58 ± 4.78	65.00 ± 4.77	67.42 ± 4.34
ALB (g/L)	31.32 ± 3.81	30.01 ± 3.70	30.16 ± 4.35
TGlob (g/L)	37.00 ± 5.23 [ab]	34.97 ± 5.24 [b]	37.25 ± 6.33 [a]
α1-Glob (g/L)	3.37 ± 0.33 [a]	3.00 ± 0.34 [b]	3.12 ± 0.47 [b]
α2-Glob (g/L)	10.51 ± 2.02 [b]	11.02 ± 2.37 [ab]	12.07 ± 2.79 [a]
β-Glob (g/L)	14.81 ± 2.30	14.73 ± 2.32	15.53 ± 2.21
γ-Glob (g/L)	6.95 ± 1.21 [a]	5.71 ± 0.95 [b]	6.01 ± 1.55 [b]
Different letters within row means significant differences between extractions at p<0.05			

5. DISCUSSION

5.1. CBC values

Red cells

Haematocrit

We found differences between t1 and t2, t2 being significantly higher than t1 (Table 2), but it must be stressed that all values were always within the normal limits.

There are few scientific data about the effect of fluoxetine on the haematocrit value. Haematocrit depends mainly on Red Blood Count (RBC) and the hydration state. In turn, RBC depends on the production of red cells by the bone marrow (it could be decreased or increased), their losses from the body (e.g. external haemorrhage), and their destruction in the body (e.g. haemolysis). The hydration state depends on many factors, the most important being water balance including water losses in the form of diarrhoea, vomiting, etc.[22]. We assume that all of these variables remain within normal limits in healthy animals, and therefore the haematocrit changes observed in our study would be caused by fluoxetine.

In depressed elderly human patients treated with fluoxetine[23], the haematocrit changed after 42 days, on average. The haematocrit was higher in non-respondent patients than in respondent ones, showing that haematocrit may change in response to the use fluoxetine, as has been found in our study. To the best of our knowledge this is the only article that provides some evidence concerning the relationship of fluoxetine treatment and haematocrit, although the mechanism responsible for such an effect is not known.

MCV – Mean Corpuscular Volume

We found normocytosis in t0 and t1 and macrocytosis in t2 (Reference values: 62fl – 74fl). The most common cause of macrocytosis is reticulocytosis, especially 4 – 5 days after the onset of anaemia[22]. Other causes are stomatocytosis, breed-associated stomatocytosis (e.g. poodles, miniature and standard Schnauzers, and Alaskan Malamute)[24], and artifactual swelling of RBCs in EDTA tubes during prolonged storage (from 6 to 24 hours in non-refrigerated samples, and from 24 hours in refrigerated ones)[25]. The macrocytosis of storage is common in samples mailed to laboratories or samples analysed the day after collection[22].

In our study, none of the animals had anemia or stomatocytosis.

In conclusion, it can not be ascertained if this macrocytosis was due to a storage problem or to the effect of fluoxetine. Indeed, although all samples were analysed within 48 hours after collection, we could not control whether the analyses were performed during the first or second 24 hours.

Mean Corpuscular Hemoglobin Concentration – MCHC:

MCHC is calculated using the following formula, MCHC = (Haemoglobin x 100) / Packed Cell Volume. Haemoglobin was constant between the extractions. Indirectly, we know that PCV is higher in t2 because we had macrocytosis in t2 and RBC remained constant during t0 – t2 (MCV = (PCV x 10) / RBC)[25]. Thus, if haemoglobin and RBC were constant between the three extractions, then MCHC is inversely proportional to MCV.

For that reason, low MCHC in t2 could be explained as a result of macrocytosis in t2.

White cells

Monocytes (Mono)

There are no previous studies about the effect of fluoxetine on total count of monocytes nor on the other cells of the leukogram (Neutrophils, eosinophils, lymphocyts and basophils).

A decrease was found between t1 and t2, but always within the reference values. There are no data available concerning the underlying mechanism of action, and further investigation would be needed.
In our study, this variation in monocytes does not modify the total count of white cells, perhaps due to the low decrease.

White Blood Count (WBC)

We did not find any variation in WBC. However, in one study with depressed human patients[26] the authors found that fluoxetine could have some effect on WBC, because after two months of treatment they found a decrease in WBC. In that study, the authors used WBC as an inflammatory marker, and they found that the depressed group (treatment group) had higher levels of WBC, when compared to controls in the first part of the study, whereas after two months of treatment no differences were found between groups.
In conclusion, fluoxetine could have a profound effect on WBC in animals with medical conditions or stress-related disorders due to its anti-inflammatory and anxiolytic effect, but not in healthy ones, as can be concluded from our study.

5.2. Biochemistry panel

Hepatic function-related parameters.

Fluoxetine undergoes hepatic metabolism after its absorption, being transformed into norfluoxetine (active metabolite) and a number of other metabolites[2]. CYP 450 2D6 plays a major role in its metabolism, but it is not the only enzyme involved. Fluoxetine can inhibit some of the enzymes involved in its own metabolism[27]. Moderately or highly elevated levels of aminotransferases have been observed in clinical trials in human beings[28] and in laboratory studies in rats[29]. Acute and chronic hepatitis have also been documented in human beings[30,31], and hepatic enzyme elevation has been found in 0.5% of humans treated with fluoxetine[28]. Unlike these studies, we found decreased values of transaminases (ALT and AST) after treatment with fluoxetine (without pathological significance). Nevertheless, the vast majority of these studies have no basal values, and they are clinical trials with other uncontrolled variables (patients with anxiety-related problems, or with multi-drug therapy, etc.). In one controlled study in rats[29], fluoxetine was administered orally at dosages of 8 mg/Kg and 32 mg/kg, and ALT and AST values were compared with a control group. In contrast with our study, they found that fluoxetine induced dosage-dependent liver damage. However, they had no information about basal ALT and AST. We also found a slight decrease in GGT values at t1.

In addition, no statistical differences were found in other hepatic values, neither structural nor functional (TBil, ALKP, BilA, BUN and ALB).

In conclusion, unlike all previous studies, our results reveal that there is no evidence of liver impairment due to the use of fluoxetine in healthy dogs. Nevertheless, in spite of our results, we can not advise the use of fluoxetine in dogs with liver damage, because it has not been specifically analysed in our study and further studies are needed.

Renal function-related parameters

Crea, BUN, P and Alb were included as renal function-related parameters, and no treatment effect was found in any of those parameters. There is a paucity of data regarding the effect of fluoxetine on renal function. In one study[32], the authors did a systematic review of randomized clinical trials and observational studies examining antidepressants in patients with renal failure. The authors concluded that, unlike other antidepressants, the pharmacokinetic parameters of fluoxetine were similar between patients with renal impairment and healthy controls. Although there are no other studies about the effect of fluoxetine in those parameters, our results, together with the conclusions of Nagler and colleagues[32], suggest that the use of fluoxetine in healthy animals does not affect renal function, and it could be safe even in animals with renal impairment.

Ion Panel

Na, K, Na/K, Cl and Ca were included in this panel. No statistical changes were observed with Na/K and Ca, whereas Cl decreased in the induction period and remained low during the withdrawal period, and Na increased during t0-t1 and remained high between t1-t2.

Although it is uncommon, hyponatraemia has been reported in 0.1% of humans treated with fluoxetine[2,12-15,33], especially in the elderly[15], during the first two weeks of treatment with fluoxetine. The mechanism underlying this effect is not well understood, but it has been attributed to reduced antidiuretic hormone secretion. A recent study in rats provides evidence that this decrease in the plasmatic sodium level can be attributed, at least in part, to the intrinsic capacity of fluoxetine to increase water permeability in the inner medullary collecting duct (IMCD), leading to an increase in water absorption[34]. In our study, fluoxetine leads to a slight increase in the serum sodium level (within the physiologic limits).

The differences between our data and the previous studies, increases of natraemia in contrast with hyponatraemia observed in few patients, could be due to the mean of age the dogs involved in our study (7.42 years), since they were not geriatric, and the fact that

hyponatraemia is a really uncommon side-effect. We did not observe hyponatraemia in any of the dogs studied. However, further studies are needed in order to understand the underlying mechanism of that increase.

Potassium levels remained unchanged after the treatment, as has been found in other studies[34].

Chloride decreased in t1 and t2, as compared with t0, but it was always within the physiologic limits. Further studies are needed in order to know the underlying mechanism. Evaluation of chloride concentration must be performed in conjunction with evaluation of sodium concentration. Changes in water balance alter chloride and sodium concentrations proportionately, a phenomenon known as artifactual hypochloraemia[35]. Changes in the permeability of IMCD caused by fluoxetine[34] may lead to excessive loss of chloride.

Calcium did not change during the treatment, and there are no previous data available dealing with this issue.

Thyroid function

Thyroid hormones have an important role in animal and human behaviour. Changes in thyroid hormone levels can modify many aspects of animal behaviour (e.g. sexual behaviour, level of activity, etc.).

Additionally, a relationship between thyroid function and behavioural problems has been suggested. Hypothyroidism has been associated with aggressive and fear-related behaviours in dogs[36], and with many psychiatric disorders in human patients[37,38]. Nevertheless, the mechanism underlying such effects has not been elucidated. It was therefore interesting to evaluate the relationship between the use of fluoxetine and thyroid hormone levels.

Total T4 (tT4) and TSH were analysed in order to evaluate thyroid function. None of them changed during treatment in our study. Several studies in human patients with major depression have examined the effects of selective serotonin reuptake inhibitors (SSRIs) on thyroid function and have yielded ambiguous results[39-43]. In the only prospective study that evaluates the effect of fluoxetine (and sertraline) on thyroid function in humans, 67 subjects were involved[16]. Twenty-eight patients with major depression and hypothyroidism on adequate levothyroxine therapy were randomized for treatment with fluoxetine (n=13) or sertraline (n=15); 29 patients with major depression and normal thyroid function were treated with fluoxetine (n=15) or sertraline (n=14) and 10 control

patients with hypothyroidism were put on adequate levothyroxine treatment without depression. The authors found that patients with major depression and normal thyroid function who were treated with fluoxetine demonstrated a significant reduction of T3 after 15 and 30 days of treatment, and tT4 after 15 days, 30 days and 90 days of treatment (all of the intervention period) respectively. However, all thyroid parameters remained within the euthyroid range. In the control group and in the group of depressed patients with primary hypothyroidism, no changes were observed in T3 and tT4. TSH did not change in any group. Our results support part of the conclusions of this study. Fluoxetine does not change TSH levels and, in our case, tT4 concentrations.

Glycaemic-related parameters

Both glucose and fructosamine blood concentration undergo significant statistical changes during (t1) and after (t2) treatment. In a review article[44], the authors analysed 17 published case reports of glucose dysregulation associated with antidepressant agents. They concluded that hypoglycaemia is associated with fluoxetine and other antidepressants with an affinity for serotonin reuptake transporter (e.g. SSRIs and clomipramine). We observe the same dysregulation in glucose levels, which decrease in t1 and increase after treatment (t2). However, the levels of fructosamine undergo the opposite change in our study. Fructosamine increases during treatment (t1) and decreases at the end of the withdrawal period (t2). Fructosamine levels correlate with the glucose blood levels during the preceding two to three weeks[45] and is not affected by acute increases in blood glucose concentrations, as occurs with stress hyperglycaemia.

Long-term use of SSRIs is associated with an increased risk of diabetes[46-48]. This could be attributed to weight gain, a frequent side-effect of treatment with SSRIs. Weight gain that leads to obesity is associated with an increased incidence of hypertension, dyslipidaemia, coronary artery disease, insulin resistance and overt diabetes in humans[49]. Despite these findings little is known about the pathophysiology of SSRIs as direct inducers of insulin resistance. A recent study[49] demonstrates that SSRIs induce insulin resistance in cultured Min6 cells and isolated murine islets. That result could explain why fructosamine remained high during treatment in dogs.

The differences between serum levels of glucose and fructosamine observed in our study, and seen in other studies in human beings separately, could be due to the fact that fructosamine remains unaffected by other circumstances that modify acute concentration of glucose.

From a clinical point of view, glucose levels should be carefully monitored when administering fluoxetine, particularly in diabetic animals, due to its potential effect on glucose metabolism and insulin resistance.

Lipid-related parameters

As mentioned above, the use of SSRIs is associated with weight gain, which could lead to dyslipidaemia in human beings. There are two clinical cases in the literature of severe and moderate hypertriglyceridaemia secondary to citalopram and fluoxetine[50] and to venlafaxine and fluoxetine[51] in human medicine. One retrospective study reports a correlation between the use of fluoxetine, sertraline or fluvoxamine (n=131) and abdominal obesity and hypercholesterolaemia[46]. The authors concluded that patients taking SSRIs should be carefully monitored for obesity and dyslipidaemia. In our study, no significant statistical differences were found in cholesterol and triglyceride serum levels. The differences between these human studies and our report could be explained in three different ways. The length of the study, the number of dogs involved in conjunction with the fact that a severe or moderate dyslipidaemia is a rare side-effect and, finally, it could be that the effect of fluoxetine on lipids in dogs is different from human beings. Further studies are needed in order to clarify this.

Finally, these results are especially important because some behavioural changes have been associated with variations in lipid profiles, such as Asperger syndrome[19], generalized anxiety[18], major depression[17], and bulimia nervosa[52] in human beings, and obsessive-compulsive disorders in both humans and animals[20,21].

Moreover, the fact that fluoxetine does not modify the cholesterol level is important for three additional reasons. First, adequate levels of cholesterol are crucial for serotonin metabolism and myelination in the brain[19]. Second, cholesterol is required for the development of serotoninergic CNS neurons and for the catabolism and transport of serotonin[19]. Finally, studies in humans indicate a positive correlation between cholesterol and serotonin levels[53].

Others – Creatine Kinase

CK increases after 45 days (mean=80.67 IU/L) of treatment and remains significantly higher at 60 days (mean=73.33 IU/L). These changes had no clinical evidence because they moved into the physiological range (69 IU/L – 309 IU/L). CK is a sensitive indicator of muscle damage and only large increases (>10,000 IU/L) or persistent increases, even if moderate (>2,000 IU/L), are generally of clinical significance[54]. One study evaluates the

effect of fluoxetine on CK activity in the brain after 28 days of treatment, but not in muscle or heart[55], nor serum concentration. The study mentioned found that after 28 days of treatment, the CK activity decreases two hours after the last injection of fluoxetine but not after 24h. However, in dogs and horses they did not find a relationship between WBC counts, serum CK, or cerebrospinal fluid (CSF) total protein and CSF CK[56,57]. In fact, it is known that CK, a large macromolecule, does not cross the blood-brain-barrier (BBB); therefore, increased activity of this enzyme in CSF is considered to be of CNS origin (if the BBB is intact). Thus, it is likely that these slight changes in serum CK due to the use of fluoxetine do not directly affect a dog's behaviour.

To the best of our knowledge, there are no other studies that evaluate the effect of fluoxetine on CK serum concentration. The most important conclusion is that fluoxetine may change CK serum concentration, but further studies are needed in order to clarify the exact relationship and the underlying mechanism.

Coagulation profile

The coagulation profile included prothrombin time (PT), activated partial thromboplastin time (aPTT) and thrombin time (TT). Differences were found only in PT time. PT increased after 45 and 60 days of treatment, but always within the physiologic range. Because bleeding abnormalities are common among patients treated with fluoxetine, many studies have been performed. The general conclusion is that decreased platelet aggregation and activity, and prolongation of bleeding time (primary haemostasis) are common, but modification of platelet count, PT, aPTT and TT (coagulation cascade) are much less frequent[58]. In fact, other studies[59,60] evaluated the effect of fluoxetine on PT, aPTT and TT (among others), and no differences were found in any case.

To conclude, bleeding abnormalities in association with the use of fluoxetine are common. These abnormalities are more likely to be due to primary haemostasis alterations more than to changes in the coagulation cascade.

Total protein test

We found no changes in total protein serum and albumin concentration. Significant results were found in total globulin serum. This change responds to significant statistical variations in α1-glob that decreased during and after treatment and in α2-glob, which were higher at the end of the study (Day 60). γ-Glob decreased at 45 and 60 days of study, but only in females. Variations in α1-glob and γ-Glob were always within the physiologic range and were minor changes. Although in the case of α2-glob the variations were minor

too, the means of concentrations were slightly higher than was the physiologic range in each sample. Means were 10.51 g/L, 11.02 g/L and 12.07 g/L in T0, T1 and T2, respectively, the normal range being 4.6 – 9.9 g/L. We did not find any controlled factor that could explain that results. Otherwise, the variations were minor in all cases, and more studies would be needed in order to corroborate our findings with α2-glob.

There are few studies in the literature that have evaluated the effect of antidepressant drugs on serum proteins. Van Hunsel et al. (1996)[61] found differences in the major, electrophoretically separated protein fractions (α1-glob, α2-glob and γ-Glob) between human patients with major depression and the control group. They did not find differences due to the treatment with any antidepressant used in the study (fluoxetine, trazodone and pindolol). There are no studies carried out in veterinary medicine or in human patients without psychiatric disorders.

6. CONCLUSSIONS

We can conclude that the use of fluoxetine in dogs is safe, and it does not seem to change any parameter that could affect a dog's behaviour.

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DISCUSIÓN GENERAL

1. PROBLEMAS MÉDICOS COMO CAUSA DE PROBLEMAS DE COMPORTAMIENTO

1.1. Revisión bibliográfica

Los problemas médicos pueden desencadenar y/o emporar problemas de comportamiento. La relación entre ambos tipos de problemas está ampliamente documentada tanto en medicina humana como en medicina veterinaria (capítulo 1). Sin embargo, en términos generales, ambas fuentes bibliográficas (medicina humana y medicina veterinaria) difieren en dos puntos clave. En primer lugar, la evidencia científica en medicina humana es más abundante. En segundo lugar, en medicina humana las evidencias de causalidad, para la mayoría de los problemas que hemos analizado en el capítulo 1, son mayores que las disponibles en medicina veterinaria. Aunque con alguna excepción significativa (p.ej., Niimi et al., 1999; Hejjas et al., 2009; Vermeire et al., 2009a; Vermeire et al., 2009b; Amat et al., 2013), los estudios que relacionan en medicina veterinaria ambos problemas suelen ser epidemiológicos o basados en casos, más que en estudios prospectivos.

Estas diferencias pueden ser debidas a tres motivos: en primer lugar (1) porque algunos aspectos de los problemas de conducta (psiquiátricos) en medicina humana son más fáciles de documentar gracias al lenguaje verbal. En segundo lugar (2) por la disponibilidad de recursos tecnológicos y económicos para llevar a cabo los estudios y, finalmente, (3) porque algunas de las técnicas más útiles para poder establecer una relación funcional entre el SNC y el comportamiento observado son, a día de hoy, más difíciles de usar en medicina veterinaria. Dos ejemplos podrían ser la tomografía de emisión de positrones (PET, del inglés *Positron Emission Tomography*), y la tomografía computerizada de emisión monofotónica (SPECT, del inglés *single photon emission computed tomography*). Las bases técnicas para obtener la imagen en las PETs y las SPECTs son diferentes, pero la finalidad es estudiar la función biológica del tejido analizado. Se utilizan en muchos campos, especialmente, para medir la actividad biológica de diferentes tejidos en oncología, embriología y, también, en psiquiatría para evaluar el funcionamiento del cerebro bajo diferentes circunstancias. El uso de las PETs en animales de laboratorio es relativamente común, especialmente desde que se han desarrollado PETs con de alta definición, por lo que pueden usarse en animales de pequeño tamaño (animales de laboratorio como ratas y ratones)(Cherry y Gambhir 2001). Sin embargo, aunque hay estudios con PETs en multitud de especies (desde ratones, pasando por los perros, hasta primates no humanos), los estudios del funcionamiento del SNC para estudiar directamente un comportamiento son muy limitados en medicina veterinaria. El

motivo es que requieren que el individuo esté dentro del arco, quieto y sin anestesia. Se han hecho algunos estudios con primates entrenados para tal fin (Tsukada et al., 2000). Sin embargo, las conclusiones que pueden extraerse de esos artículos desde un punto de vista diagnóstico clínico son, por ahora, muy limitadas.

La mayoría de estudios con PET y SPECT realizados en animales de compañía en relación a los problemas de comportamiento, han demostrado diferencias en la funcionalidad del SNC, en animales anestesiados, con problemas de ansiedad. Demostrando una disfunción de la corteza prefrontal y del sistema límbico en animales con problemas de ansiedad (Vermeire et al., 2009a) y alteraciones en la actividad serotoninérgica en diferentes regiones del SNC (Diksic et al., 1991; Peremans, et al., 2002; Vermeire et al., 2009b).

En definitiva, este puede ser un gran campo de investigación en los próximos años, ya que muchas de la patologías de comportamiento de los animales de compañía pueden servir como modelo animal para los problemas psiquiátricos en humana (lo cual ayudaría a salvar la desventaja del coste económico). La ventaja que otorgan estas técnicas es que pueden realizarse estudios de actividad de neurotransmisores, receptores, actividad de áreas cerebrales, etc. repetidas veces sobre el mismo individuo sin tener que eutanasiarlo. Las desventajas principales son el coste económico y la dificultad de realizarlas con el animal despierto.

1.2. Problemas médicos como causa de problemas de comportamiento

Desde un punto de vista práctico, lo que se extrae de la revisión bibliográfica es que existen algunos de estos problemas médicos suponen un verdadero reto diagnóstico. En algunas patologías el único signo clínico observable son los cambios en el comportamiento. La mayoría de los problemas comentados en el capítulo 1 abren un amplio abanico de estudio para el futuro. Además, de nuevo, el estudio de estas alteraciones en los animales pueden arrojar información para mejorar los diagnósticos y tratamientos de problemas iguales o similares en medicina humana.

En primer lugar, será de especial interés estudiar el efecto que puede tener la privación o falta de sueño sobre la sensibilidad al dolor en los animales. Esta relación causal que está muy bien establecida en medicina humana, es prácticamente desconocida en animales de compañía. Es especialmente importante por los siguientes motivos: (1) porque algunos problemas de comportamiento inducen cambios en el ciclo de sueño-vigilia. La disfunción cognitiva es un problema muy prevalente en perros y gatos geriátricos. En un estudio hecho en España con 270 perros de más de 7 años, el 74%, 19.8%, 4.6% y el 1.3% de los

propietarios decían que sus perros tenían, respectivamente, 1, 2, 3 o 4 signos compatibles con síndrome de disfunción cognitiva (Mariotti et al., 2009). Uno de los síntomas de la disfunción cognitiva es la alteración del ciclo sueño-vigilia (Landsberg et al., 2011). Se debe tener en cuenta que tanto los perros como los gatos geriátricos tienen más probabilidad de tener dolor que los animales jóvenes (Muir III et al., 2004). Por lo tanto, sería muy interesante poder estudiar el efecto que tiene la falta de sueño en los perros y gatos sobre la sensibilidad al dolor. Podría ayudar a mejorar el bienestar de los animales geriátricos. (2) Si finalmente se demuestra esta relación en medicina veterinaria, podrían cambiar algunos protocolos de hospitalización o, incluso, las propias instalaciones de hospitalización. En general, aunque no existen datos científicos que lo corroboren, las instalaciones y los protocolos de hospitalización tienen poco en cuenta el descanso de los animales ingresados. La mayoría de las salas disponen de luz artificial que está en marcha la mayor parte del día, ruidos de bomboa de infusión, movimiento del personal en la sala, etc. que podría dificultar el descanso de los animales hospitalizados. Esta falta de descanso podría estar, por un lado, dificultando la recuperación del animal (por la presencia de más dolor) y/o, por otro, favoreciendo el uso innecesario de medicación analgésica.

En segundo lugar, se evidencia que existen campos de estudio completamente nuevos y que pueden ser de relevancia clínica destacable. Las patologías que pueden afectar al órgano vomeronasal (VNO) están muy poco estudiadas. Puesto que las feromonas tienen como función inducir cambios de conducta en el individuo receptor, y el VNO se encarga de percibirlas, parece lógico pensar que alteraciones en su función puedan afectar significativamente el comportamiento. Las única evidencia que existe por ahora es que el VNO puede mostrar un infiltrado inflamatorio (Verin et al., 2010). Las investigaciones futuras deberían ir encaminadas en tres aspectos clave. Primero (1) correlacionar los cambios histopatológicos con cambios de comportamiento en el individuo, a ser posible con estudios prospectivos con grupo control. Segundo (2) investigar cómo realizar su diagnóstico *in vivo*, a ser posible con técnicas poco invasivas (radiológicas, resonancia magnética nuclear, citologías, etc.) y, tercero, (3) posibles indicaciones terapéuticas que reviertan los signos clínicos e histopatológicos.

Finalmente, el capítulo 1 evidencia la necesidad de ahondar más en el estudio de todos aquellos problemas neurológicos que alteran el comportamiento sin que haya cambios estructurales del SNC, sino únicamente cambios funcionales. Queda demostrado tanto en medicina humana, como en algunos modelos animales con ratas, que los traumatismos craneoencefálicos pueden provocar cambios de comportamiento. Estos cambios se dan incluso sin la presencia de lesiones en las pruebas de imagen convencional (sí puede haberlas en pruebas de nueroimagen funcional). De producirse un proceso similar en

perros y gatos (es razonable pensar que sea así), se abriría un campo muy importante de colaboración entre neurólogos y etólogos. Sobre todo si se tiene en cuenta que los traumatismos craneoencefálicos son frecuentes (representan el 25% de los traumatismos severos) (Simpson et al., 2009). Lo mismo podría suceder con las isquemias transitorias u otras patologías similars.

Durante mucho tiempo, en medicina humana, la psiquiatría y la neurología han sido disciplinas completamente separadas. Sin embargo, a la luz de los conocimientos actuales, esta separación parecer ser completamente arbitraria y contraproducente (Martin 2014). La neuropsiquiatría, que es la rama de la medicina que estudia los cambios de comportamiento debidos a problemas neurológicos, ha surgido con fuerza estos últimos años. En un artículo al respecto, Price et al. (2000) aseguran que:

The education of future psychiatrists and neurologists should be redesigned....Both disciplines should emphasize basic neuroscience, genetics, neuroanatomy, neuropathology, neuroimaging, neuropsychology, cognitive neuroscience, behavioral phenomenology, neuropsychopharmacology, and psychological interventions. Neurologists in training should be given a rich clinical exposure to patients suffering from major mental and neuropsychiatric diseases. Psychiatrists in training should be given more exposure to patients with neurologic syndromes, particularly those that are likely to be accompanied by psychiatric symptoms.

Quizás, en un futuro no muy lejano, deba producirse un acercamiento similar de ambas disciplinas también en la medicina veterinaria. De hecho, el capítulo 2 representa en sí mismo este concepto.

1.3. Rompiendo la barrera entre neurología y etología

La polioencefalomielopatía espongiforme congénita es una enfermedad muy rara que ha sido descrita en diversas especies, entre ellas los gatos (Jones et al., 1992; Morita et al., 2002; Vidal et al., 2004) y los seres humanos (Jellinger y Seitelberger 1970; Janota 1974; Ropper y Brown 2005). En las personas, además de con signos neurológicos evidentes (p.ej., crisis epileptiformes), se correlaciona con graves alteraciones cognitivas (Janota 1974; Ropper y Brown 2005). En ninguno de los casos descritos en gatos (Jones et al., 1992; Morita et al., 2002; Vidal et al., 2004) se documentan detalladamente los cambios de comportamiento, ni estos son el principal motivo de consulta. Hasta el momento, en todos los casos descritos, el desenlace ha sido fatal en pocos días o semanas (en animales y humanos) o hasta pocos años en niños.

A parte de describir una enfermedad muy rara, el caso descrito en el capítulo 2 es particular por los siguientes motivos: (1) Es el único caso de polioencefalomielopatía espongiforme congénita en animales, descrito hasta el momento, en que los signos de comportamiento fueron el principal motivo de consulta. Esto no significa que el animal no mostrara signos neurológicos antes que los problemas de comportamiento, o que se iniciaran al mismo tiempo (que es probable). Lo que significa es que los primeros signos clínicos que motivaron la consulta fueron la falta de aprendizaje de los hábitos higiénicos y la mala conducta de juego de la gata. (2) La gata fue eutanasiada poco antes de los 3 años de vida, por lo tanto, el tiempo de vida fue muy superior al resto de casos descritos en gatos. (3) Hasta el momento de la eutanasia, el tratamiento conjunto neurológico-etológico proporcionó una calidad de vida aceptable tanto al animal como a los propietarios (en su relación con el animal).

Finalmente el caso constata dos evidencias. La primera, la necesidad de realizar siempre un abordaje completo y sistemático de cualquier problema de comportamiento (como el descrito en la introducción). La segunda, que la colaboración entre las diferentes disciplinas puede favorecer el bienestar tanto de los animales como de los propietarios.

1.4. Dolor y agresividad en el perro – Dos patrones diferentes de agresividad por dolor en el perro

El principal resultado que se extrae del capítulo 3 es que existen dos patrones de expresión de la agresividad por dolor. El elemento diferenciador es el hecho de que en el momento de la aparición del dolor, el animal ya hubiera mostrado agresividad con anterioridad. Las características de cada patrón se detallan en la siguiente tabla:

Grupo	Características de la agresividad
No agresivos antes del inicio del dolor	Impulsiva y se da en contextos de contacto.
Agresivos antes del inicio del dolor	No impulsiva y se observa un empeoramiento de la agresividad pre-existente.

En otras palabras, lo que parecen indicar estos resultados es que los perros que no eran agresivos antes de mostrar dolor, cuando reaccionaban agresivamente lo hacían como una forma de evitar más dolor. Simplemente para evitar que los tocaran y que esto pudiera empeorar el dolor. Es importante destacar que el perro puede aprender de esta

experiencia y, en el futuro, reaccionar de forma agresiva en un contexto igual o similar aunque ya no tenga dolor. Las aplicaciones prácticas, desde el punto de vista de prevención, son dos: (1) Se debería evitar, siempre que sea posible, las manipulaciones dolorosas (en la clínica o en el entorno familiar). La utilización de analgesia y sedaciones en el ámbito del manejo clínico es necesario para que las visitas posteriores no sean problemáticas. Se deberá educar al propietario a realizar un correcto manejo en casa de las situaciones que pueden resultar dolorosas (como administración de tratamientos óticos, oftálmicos, corte de uñas, etc.) para evitar accidentes y mejorar el bienestar del animal. (2) Los cursos de socialización deberían incluir un plan para habituar a los perros a dichas manipulaciones dolorosas.

Por otro lado, lo que se observó en los perros que ya eran agresivos cuando empezaban a manifestar dolor es que empeoraban la agresividad pre-existente. Este hecho no implica que los perros de este grupo no reaccionaran de forma agresiva e impulsiva cuando si se sintieran amenazados porque alguien fuera a manipular la zona dolorosa. Es decir, los más probable es que estos perros se mostraran más agresivos en las situación en las que ya lo eran anteriormente porque el dolor aumentara su irritabilidad (disminuyera su umbral de agresividad), pero que al mismo tiempo también fueran agresivos e impulsivos si alguien les intentara tocar la zona dolorosa. Lo que parece más probable es que los propietarios de perros agresivos sean más reticentes a la hora de manipularlos.

En definitiva, el empeoramiento de un problema de agresividad puede ser el único síntoma de que un animal tiene dolor. Por lo tanto, siempre deberán buscarse puntos de dolor en animales que sufran un empeoramiento de un problema de agresividad. Esto refuerza la necesidad de colaboración entre diferentes disciplinas.

2. PROBLEMAS DE COMPORTAMIENTO COMO CAUSA DE PROBLEMAS MÉDICOS

2.1. Relación entre los problemas de comportamiento y la alteración de los parámetros hematológicos y bioquímicos en el perro

Existe una amplia evidencia que el estrés puede afectar a la salud tanto en medicina humana como veterinaria. En la literatura científica se encuentran numerosos estudios realizados con diferentes tipos de eventos estresantes (imposibles de enumerar todos ellos), como por ejemplo el estrés inducido por el ejercicio físico, estrés social, estrés causado por experiencias traumáticas (como una guerra o maltratos), etc. Muchos de estos estudios demuestran que el estrés tiene consecuencias sobre la fisiología del animal. Otros estudios en personas demuestran que técnicas, como el yoga (Carranque et al., 2012), que tienen como objetivo disminuir el estrés, también pueden modificar los parámetros fisiológicos de las personas que lo practican.

Los problemas psiquiátricos, en medicina humana, como fuente de estrés, también han sido estudiados. De nuevo, existe una amplia evidencia sobre el efecto que pueden tener dichos problema sobre el recuento de glóbulos blancos, el volumen de las plaquetas, o diferentes parámetros bioquímicos como el panel lipídico, glucosa, proteínas, etc. (Boscarino y Chang 1999; Miller et al., 2013a; Miller et al., 2013b; Semiz et al., 2014). Estos indicadores hematológicos pueden usarse, en ocasiones, para realizar un pronóstico de la evolución de determinadas patologías como, por ejemplo, el ratio neutrófilos/linfocitos (Horne et al., 2005; Kaneko et al., 2012; Lee et al., 2012; Tomita et al., 2012).

Aunque existen evidencias de que un proceso similar ocurre en perros, la evidencia de que los problemas de comportamiento en el perro, como fuente de estrés, puedan hacerlo es mucho más limitada. Los estudios en perros se han centrado, sobre todo, en el efecto que tienen los problemas de agresividad sobre el estado tiroideo y lipídico del animal (Pentürk y Yalcin 2003; Civelek et al., 2007; Carter et al., 2009; Radosta et al., 2012).

Los resultados del cuarto capítulo muestran que: (1) los tres problemas de comportamiento estudiados pueden modificar los parámetros fisiológicos del perro y (2) que los diferentes problemas de conducta, considerados como una posible fuente de estrés, alterar de forma diferente la fisiología del animal.

Los tres problemas de comportamiento estudiados (agresividad, miedo y ansiedad por separación) modifican algunos de los parámetros hematológicos y bioquímicos usados rutinariamente en la clínica diaria para evaluar el estado de salud del perro. Sin embargo,

estos cambios son, en prácticamente todos los casos, variaciones de la media dentro de los rangos de referencia y, por lo tanto, con poco significado clínico. Sin embargo, es importante destacar tres de estos cambios: (1) la concentración de ALT en problemas de ansiedad por separación, (2) la concentración de colesterol total en perros con agresividad y, sobre todo, (3) el ratio neutrófilos/linfocitos en perros también con agresividad. La ALT en los perros con ansiedad por separación está significativamente más elevada. Sin embargo, aunque la media poblacional de perros con ansiedad por separación está dentro de los valores de referencia, si se tiene en cuenta la desviación estándar, algunos individuos podrían salirse de dichos valores. Este hecho tiene varias lecturas. En primer lugar, que el estrés causado por la ansiedad por separación podría tener algún efecto a nivel de estructura hepática, puesto que es una enzima que se encuentra mayoritariamente en el citoplasma de los hepatocitos. Es importante tenerlo en cuenta ya que la mayoría de fármacos usados para controlar los signos clínicos de la ansiedad por separación son de metabolización hepática (Beaver 2009). Sin embargo, estos resultados deben tomarse con cautela, puesto que el número de animales del estudio con ansiedad por separación es bajo (n=10). Para poder establecer, finalmente, una relación entre ambos sucesos se debería proponer un estudio con un mayor número de animales y que fuera un estudio de casos-control es decir, que las poblaciones de ambos grupos (con y sin ansiedad por separación) tuvieran características similares en cuánto a edad, raza, estado reproductivo, etc. El segundo parámetro a tener en cuenta es el colesterol total en perros con agresividad. A diferencia de los estudios realizados hasta el momento, tanto en perros (Pentürk y Yalcin 2003; Civelek et al., 2007) como en personas (Chen et al., 2001), en nuestro caso se observa un aumento de la concentración total de colesterol. Sin embargo, esta diferencia podría venir dada por la naturaleza de nuestro estudio en comparación con los otros realizados en perros. En el caso de la agresividad en nuestro estudio, hace referencia a una expresión del comportamiento. En otras palabras, hace referencia al hecho de ser agresivo y no a ninguna agresividad en concreto. Los estudios previos estaban realizados sobre un diagnóstico concreto de agresividad (la agresividad por dominancia y la agresividad inducida por aprendizaje). Por lo tanto, una hipótesis podría ser que cada tipo de agresividad podría tener un efecto diferente sobre la concentración de colesterol. La diferente naturaleza de los estudios, hace que sean difícilmente comparables entre sí.

El valor que podría tener una mayor relevancia clínica es el ratio neutrófilos/linfocitos. El ratio neutrófilos/linfocitos es un buen indicador de estrés tanto en medicina humana como veterinaria. Se ha visto que, desde un punto de vista fisiológico, lo que sucede es que el cortisol liberado como consecuencia de la respuesta de estrés favorece, por una parte, la

adherencia de los linfocitos a las paredes del endotelio vascular y su paso al interior de las células de diferentes órganos como ganglios linfáticos y bazo sobre todo y, por otra, la liberación de neutrófilos a la sangre (Bishop et al., 1968; Cohen 1972; Dhabhar 2002). Esto se traduce en que altos niveles de cortisol aumentan el ratio neutrófilos/linfocitos circulantes. Quedan muchas respuestas por resolver al respecto del significado que puede tener este indicador. Aunque da información sobre si un determinado individuo está sometido a más o menos estrés en relación a otro individuo, poco se sabe sobre la capacidad inmunológica de ese individuo. Es decir, el aumento del ratio indica que el animal ha estado sometido estrés, o que ha sufrido un proceso inflamatorio o infeccioso. Sin embargo, ¿indica esto que el animal tiene una menor capacidad de enfrentarse a dicho proceso estresante o inflamatorio o todo lo contrario? (Davis et al., 2008). Quizás el hecho de poder responder así le otorgue al animal alguna ventaja a la hora de afrontar dichos eventos. Parece obvio que se requieren más estudios para poder determinar el valor real de este indicador. Por otro lado, múltiples estudios en medicina humana utilizan este indicador como parámetro fisiológico de bajo coste para determinar el pronóstico de muchas alteraciones médicas. Parece ser que ratios altos de neutrófilos/linfocitos son indicadores de riesgo alto cardiovascular y de mal pronóstico de algunas cirugías vasculares y algunos tumores de colon, cervicales, pulmonares y de mama, entre otros (Horne et al., 2005; Kaneko et al., 2012; Lee et al., 2012; Tomita et al., 2012). Por lo tanto, parece ser que los problemas de comportamiento estudiados no inducen directamente problemas fisiológicos que puedan desencadenar en una patología en el perro. Sin embargo, el incremento del ratio neutrófilos/linfocitos provocado por los problemas de agresividad podría tener un papel importante en la salud del perro, y sería un buen campo de estudio futuro.

2.2. Relación entre el tratamiento con fluoxetina de los problemas de comportamiento y la alteración de los parámetros hematológicos y bioquímicos en el perro

Como se comentó en la introducción, existen diferentes estrategias de tratamiento para corregir los problemas de comportamiento en el perro. El uso de psicofármacos está indicado en muchos casos. Animales con problemas de miedo o fobia, ansiedad, trastornos compulsivos y agresividad, entre otros, pueden beneficiarse de ellos. Tanto los problemas en sí, como su tratamiento, suelen tener un curso crónico, lo que se traduce que muchos de estos perros acaban tomando medicación durante meses o años.

El fármaco antidepresivo más utilizado en medicina veterinaria es la fluoxetina. Se usa, sobre todo, para problemas de agresividad, especialmente si es impulsiva, trastornos compulsivos y problemas de ansiedad por separación (Simpson y Papich 2003; Beaver 2009). Hay muchos estudios sobre el papel que tiene la fluoxetina sobre los parámetros fisiológicos en medicina humana y animales de laboratorio. Sin embargo, el papel que tiene sobre la fisiología del perro han sido estudiados en menor medida.

Aunque existen variaciones en algunos de los parámetros fisiológicos estudiados en el capítulo 5, todas las variaciones observadas son mínimas y están dentro de los parámetros fisiológicos. Esto nos conlleva a diferentes conclusiones prácticas. La primera es que (1) la fluoxetina parece ser un fármaco seguro en perros. La segunda (2) que al no modificar los parámetros tiroideos parece un fármaco seguro para animales con hipotiroidismo y problemas de comportamiento y, finalmente, (3) que variaciones que puedan presentarse en dichos parámetros durante el tratamiento con fluoxetina de un perro, deberían ser tomados en consideración como indicador de enfermedad. En otras palabas, si aparecen alternaciones hematológicas o bioquímicas fuera de los valores de referencia durante el tratamiento con fluoxetina se debería investigar la causa subyacente, puesto que la probabilidad de que se deba al tratamiento farmacológico es baja.

Finalmente, en un futuro, sería interesante poder analizar si los cambios en los parámetros bioquímicos y hematológicos debidos a los problemas de comportamiento (mencionados en el capítulo 4) podrían ser revertidos con el tratamiento. Como se vio en la introducción general, el tratamiento de los problemas de comportamiento consta de diferentes partes. Normalmente, el tratamiento se basa en técnicas de modificación de conducta (mediante el aprendizaje). Los psicofármacos suelen ser un coadyuvante del tratameinto. Por lo tanto, conocer el impacto del tratamiento farmacológico sobre los parámetros fisiológicos del animal (capítulo 5), es necesario para esclarecer si el tratamiento basado en el aprendizaje es capaz de modificar las alteraciones vistas en dichos parámetros (capítulo 4).

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CONCLUSIONES

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1. Algunos problemas médicos del perro y el gato cursan con un cambio de comportamiento como único signo clínico de la enfermedad. Incluso, pueden hacerlo sin alterar los parámetros fisiológicos y/o las pruebas de imagen.

2. En los perros el dolor induce dos patrones diferentes de agresividad en función de si eran agresivos antes del inicio del dolor. Los perros que no son agresivos antes de que se inicie el proceso doloroso son más impulsivos y la agresividad se da en un contexto de manipulación. Los perros que son agresivos antes del inicio del dolor empeoran la agresividad pre-existente y son menos impulsivos.

3. La ansiedad por separación, los problemas de miedo y los problemas de agresividad cambian los parámetros hematológicos y bioquímicos del perro.

4. La ansiedad por separación en el perro aumenta la concentración de ALT sérica. Los problemas de agresividad en el perro aumentan la concentración de colesterol total y el ratio neutrófilos/linfocitos, lo que podría relacionarse indirectamente con problemas en la salud del perro, y no modifican las concentraciones de T4 total y TSH.

5. La fluoxetina modifica algunos parámetros hematológicos y bioquímicos del perro pero es segura para el tratamiento de problemas de comportamiento en el perro.