DANIELA RAMOS

**Evaluation of medical, behavioural and
physiological aspects of domestic cats
(*Felis catus*) with inappropriate urination**

São Paulo
2012

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Tese apresentada ao Programa de
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Prof. Dr. Archivaldo Reche-Junior

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CERTIFICADO

Certificamos que o Projeto intitulado "Avaliação de aspectos médicos, comportamentais e fisiológicos em gatos domésticos (*Felis catus*) que manifestam micção inapropriada", protocolado sob o nº 1457/2008, utilizando 50 (cinquenta) gatos, sob a responsabilidade do Prof. Dr. Archivaldo Reche Júnior, está de acordo com os princípios éticos de experimentação animal da Comissão de Ética no uso de animais da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo e foi aprovado "ad referendum".

We certify that the Research "Evaluation of medical, behavioural and physiological aspects of domestic cats (*Felis catus*) with inappropriate urination", utilizing 50 (fifty) cats, protocol number 1457/2008, under the responsibility Prof. Dr. Archivaldo Reche Júnior, agree with Ethic Committee in the use of animals of the School of Veterinary Medicine and Animal Science of University of São Paulo and was approved "ad referendum" meeting.

São Paulo, 22 de março de 2012.

Denise Tabacchi Fantoni
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Cesar

Twelve years ago, when the perspective of a vet career was not fulfilling me and I was about to give up on everything, you opened me a window to a fascinating world: the secret life of animals. Since then, we haven't stopped in our incredible journey exploring the mind of our canine friends. We researched together, we laughed together, and together we took "incredible" 8 years to publish our so expected Sofia's paper! The first step had been completed, but there was a lot to come. Your enthusiasm, geniality and partnership have been strongly missed. Your great lessons will be kept fresh in my mind and heart whilst carrying on doing research with joy, dedication and creativity as you always taught. You are not here to celebrate this moment but whenever you are please receive my special thanks for everything. We certainly talked a lot about canine behaviour in the past decade – all that stuff surely warmed me up to go decipher cats too!

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"Sometimes I still find myself sadly surprised by people's insensitivity to the emotional life of animals. For a long time, I wondered whether I was in the right direction, considering that my love, appreciation and empathy for animals made me feel uneasy towards the objective, "organic", and sometimes cold views of the orthodox veterinary medicine.

But when my feelings and thoughts found under the strong and sustainable "arms" of the science of animal behavior and welfare, I learned that veterinary medicine can indeed be multifaceted. It is possible to be a veterinarian and love animals so intensely to the point of desiring to uncover their mind and to listen to their hearts, understand how they think and feel, how they understand us, and what they need to be, in fact, happy.

Science has revealed crucial links between the brain and other essential systems, thus if not by pleasure, necessity has led veterinarians to open their eyes to what is hard but amazing to investigate: the behaviour of animals. To all those who, like me, have the conviction that animals are very capable of thinking and feeling, I invite you to know the foundation for our argument in favor of them: the fantastic findings of ethology, the science of animal behaviour. Veterinary medicine is lacking in people like you! And animals thank for you to exist! "

(Daniela Ramos)

Abstract

Ramos, D. **Evaluation of medical, behavioural and physiological aspects of domestic cats (*Felis catus*) with inappropriate urination.** [Avaliação de aspectos médicos, comportamentais e fisiológicos em gatos domésticos (*Felis catus*) com micção inapropriada]. 2012. 177 f. Tese (Doutorado em Ciências) - Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2012.

Commonly reported refractoriness in feline cases of inappropriate urination, in spite of well established diagnosis schema and therapeutic regimens, emphasizes the need for reviewing traditional concepts and conducts. A case-control study with 18 “sprayer” and 23 “toileter” cats, and their respective controls from the same multi-cat households (3-9 cats), was performed to investigate the following aspects: disease, stress, personality, emotionality and social behaviour. A high prevalence of medical problems, including some alterations of the upper urinary tract, was equally found among “sprayers” and “toileters”. Cystoscopy of female participant cats led to a probable diagnosis of interstitial cystitis in one “sprayer” and one “toileter”. Unreliable values concerning plasma bioamines restricted our understanding of the cats emotionality, but faecal glucocorticoid levels indicated that cats from *spraying* households (“sprayers” and their controls) were more aroused (probably more stressed) than cats from the *toileting* households. Cat owners were not consistent in their opinions with regard to personality but their more direct observations in respect to inter-cat relationships indicated that neither *spraying* nor *toileting* was associated with cats’ social behaviour or status. About recorded behaviours, no differences were seen between “cases and “controls” from either of the groups. However, proportionally more time spent moving (as opposed to stationary) by cats from *spraying* houses might have reflected the higher arousal states of the former. Results reiterate the importance of medical check-ups in any case of inappropriate urination. Furthermore, they suggest *spraying* as a feline attempt to cope in highly stressful environments.

Key words: Feline. Housesoiling. Stress. Disease. Behaviour.

Resumo

Ramos, D. **Avaliação de aspectos médicos, comportamentais e fisiológicos em gatos domésticos (*Felis catus*) com micção inapropriada.** [Evaluation of medical, behavioural and physiological aspects of domestic cats (*Felis catus*) with inappropriate urination]. 2012. 177 f. Tese (Doutorado em Ciências) - Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2012.

Relatos comuns de refratariedade em casos de micção inapropriada em gatos, a despeito do esquema bem estabelecido de diagnóstico e regimes terapêuticos, enfatiza a necessidade de rever os conceitos tradicionais e condutas. Um estudo do tipo caso-controle com 18 gatos "sprayers" (manifestadores de marcação urinária) e 23 "toileters" (exibidores de micção fora da caixa sanitária), e seus respectivos controles oriundos das mesmas residências de múltiplos gatos (3-9 gatos), foi realizado para investigar os seguintes aspectos: doença, estresse, personalidade, emotividade, e comportamento social. A alta prevalência de problemas médicos, incluindo algumas alterações do trato urinário superior, foi igualmente encontrada entre os "sprayers" e "toileters". Cistoscopia realizada em fêmeas participantes da pesquisa levou ao provável diagnóstico de cistite intersticial em uma gata "sprayer" e uma "toileter". Valores não confiáveis de bioaminas plasmáticas restringiram nossa compreensão da emotividade dos gatos, mas os níveis de glicocorticóides fecais indicaram que os gatos das casas "spraying" ("sprayers" e seus controles), mostraram-se mais agitados (provavelmente mais estressados) do que os gatos das casas "toileting". Os proprietários não se mostraram consistentes nas suas opiniões em relação à personalidade dos seus gatos, mas suas observações diretas a respeito das relações gato-gato indicaram que nem o "spraying" tampouco o "toileting" mostrou-se associado ao comportamento social e status do gato. Em relação aos comportamentos registrados em vídeo, não foram observadas diferenças entre "casos e "controles" em nenhum dos grupos. Entretanto, o maior tempo gasto movendo (proporcionalmente ao em estado estacionário) por gatos das casas "spraying" em comparação com aqueles das casas "toileting" pode ter refletido os estados mais

elevados de excitação dos primeiros. Os resultados reiteram a importância dos “check-ups” médicos em qualquer caso de micção inapropriada. Além disso, sugere-se a marcação urinária como uma tentativa do felino para lidar em ambientes altamente estressantes.

Palavras-chave: Felino. Eliminação inapropriada. Estresse. Doença. Comportamento.

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Introduction

1 INTRODUCTION

1.1 The modern cat and the human-cat relationship

After historical periods oscillating from being appreciated to persecuted, domestic cats have come to occupy a privileged position within many modern societies: they are now becoming the most popular of the companion animals. In several countries (and not exclusively within western societies as it used to be – TURNER; BATESON, 2000) the number of cats is greater than dogs (EUROMINITOR, 2008). In other countries such as Brazil where the dog population is bigger (i.e. 34.3 million dogs versus 18.3 million cats) and more dogs than cats are acquired annually as pets, the cat acquisition rate is growing faster (PEDROSA, RIBEIRO; PIRES, 2011). Therefore, even in such places, forecasts are that cats will probably outnumber dogs in the near future.

To the casual observer, domestic cats seem to adapt very well to the contemporary human lifestyle of rushing around, information overload and individualism, which lead a lack of time to spend with others, including pets. Cats seem to accept better small houses and long hours on their own; they do not demand daily walks and, with just a few exceptions, they are “naturally” toilet trained. The hard work of training an animal, which forms the basics of living with such closeness to animals is generally not a need when you own a cat. Furthermore, domestic cats have certain characteristics such as rubbing against people’s body, a relative easiness for being picked up onto a lap and climbing skills, which allow them to reach the human’s face, which helps to build a unique relationship with people (DOWNEY; ELLIS, 2007). Cats do not seem to be as mysterious and distrustful to humans as in the past and part of this is certainly the result of scientific advances in the field of animal behaviour.

Independency and resilience are probably the key features of the cats' nature, responsible for fitting them so well into modern society; their welfare and its impact on the harmony of the human-cat relationship depends upon the care and attention provided by humans though (TURNER; BATESON, 2000). In fact, in spite of the apparent suitability of cats for living with people, the current human-cat relationship has been shown not to be perfect. According to Voith (1985), 47% of 800 surveyed American cat owners complained about an undesirable behaviour shown by their cat. The number of owners identifying a cat behavioural problem reached 82% in another similar survey in USA (MORGAN, 1996). One could argue that these figures represent a mere observation by the owners about a behaviour shown by their cats. However, the fact that owners' complaints about inappropriate behaviours also appear in many cases of feline relinquishment (e.g. 47% in the research by SALMAN et al., 2000) suggests that they are likely to reflect a true dissatisfaction on the owners' part.

From a feline perspective, the chances are that cats are equally unsatisfied. People are responsible for providing cats with food, play, exercise, health care, for composing the cat groups within multi-cat houses, for deciding about their reproductive availability, their access to the outside world etc. Among these, housing conditions (MCCUNE, 1992; ROCHLIZ, 1999) seems to be what most impacts their welfare. Beyond this, cat behaviour and its emotional predispositions have shown to be influenced by a range of human factors (e.g. owner age, presence of children in the family, ADAMELLI et al., 2005, LICHTSTEINER; TURNER, 2008). The question which arises then, is whether owners are good at offering what a cat needs. The growth of feline obesity worldwide, for instance, as a result of owners overfeeding their cats is an example where the answer is no. Thus, despite their behavioural plasticity (notorious for their flexibility to adjust to the different personalities, moods and interaction styles of people – TURNER; BATESON, 2000), cats certainly face challenges in their day to day lives as a popular companion. This may lead to stress, which can pass unnoticed or manifested in a form of “mimicking diseases” (STELLA, LORD; BUFFINGTON, 2011) and undesired behaviours, such as inappropriate

urination (APBC - *Association of Pet Behaviour Counsellors*, 2005). Feline behaviour suggests, at least in part, dissatisfaction too on the cat's side.

Changes in people's life brought about by modern demands have encouraged individuals to acquire pets for social support, at least in some circumstances. However, with a longer history and closer proximity with dogs (BEAVER, 2005), it is important for humans to learn what it is to be a cat and what is necessary for a happy feline. Lack of knowledge of the characteristics and requirements of pet cats has appeared prominently among cat owners who relinquished their cats to an animal shelter (PATRONEK et al., 1996). For instance, these owners often reported they had never read a book about feline behaviour and had inappropriate care expectations. Thus, understanding cat behaviour before owning a cat might be one of the requirements of a harmonious human-cat relationship.

1.2 Feline elimination behaviour: normality versus anormality

On the basis of observations with feral cats (e.g. DARDS, 1983; NATOLI, 1985; FELDMAN, 1994), it is postulated that many of the undesirable behaviours exhibited by owned cats are actually normal feline behaviours subjected to misinterpretations or a result of constraints imposed by domestic life (BOWEN; HEATH, 2005). A traditional example is aggression, commonly reported by cat owners (PALACIO et al., 2007; AMAT et al., 2008; RAMOS; MILLS, 2009), considered an important part of their behavioural repertoire, in a species lacking the social complexity of dogs (CROWELL-DAVIS et al., 2004). In a species that preserves independence, spending a great proportion of its time in isolation (BARRY; CROWELL-DAVIS, 1999), aggressive behaviour is used, among other strategies and aside from other purposes, to maintain distance, controlling when and where contact with others occurs.

When facing adversity and under stress, cats tend to show inhibition and inactivity, rather than truly "abnormal" behaviours. For example, it has been suggested that vertical marking in the form of urine deposition (also known as

spraying behaviour), is used by cats as a form of passive aggression (OVERALL, 1997; OGATA et al., 2001), which helps prevent overt physical confrontation. Containing a range of chemicals from hormones to pheromones and amino acids, such as *feniline* (HENDRIKS et al., 1995), cat urine may act as either attractants or repellents, which serve several communicative functions within spatial organization (e.g. territorial defense, reproductive attraction, individual identification etc.) (NATOLI, 1989). *Spraying* therefore, together with other forms of scent marking (FELDMAN, 1994), plays an important role in feline sociality, being particularly useful when social challenges exist, as in multi-cat households (PRYOR et al., 2001; SKERITT; JEMMETT, 1980). A further possible function of urine *spraying* is as a coping mechanism when under anxiety and distress: it can function as a reassuring behaviour for the perpetrators themselves (BOWEN; HEATH, 2005). Far from being abnormal, a cat that *sprays* over the house is thus exhibiting a natural (i.e. normal) behaviour – the inconvenience of this in our homes is a different point for consideration.

Among feral cats living in colonies, evacuation of the contents of the bladder occurs in specific areas - these are typically peripheral to the core territory, within their home ranges; other specific resting, playing and hunting areas also co-exist (NATOLI, 1985; FELDMAN, 1994) Such places are used individually, thus distant signaling through chemical marks is crucial to prevent encounters. When urinating or defecating in this way (hereafter described as *toileting*), cats typically assume a squatting position and display a scratching-burying behaviour sequence, but on rare occasions faeces and/or urine are left uncovered (FELDMAN, 1994). *Toileting* behaviour such as this is performed by kittens as young as one month old (BEAVER, 2005), and differs from *spraying* in which the classical posture includes a standing position backing up to a surface with the tail held vertically and twitching while emitting a spray of urine (FELDMAN, 1994). Concerning *toileting* behaviour performed by owned cats, the use of a specific spot (i.e. the litterbox) and large amounts of voided urine is typically described for a normal feline. However, in urine *spraying*, it is frequently said that smaller quantities are spread on several vertical

surfaces around the house (DEHASSE, 1997; HORWITZ, 2002; BEAVER, 2005; NEILSON, 2004 a,b; BOWEN; HEATH, 2005).

Despite the above mentioned distinction of *spraying* on the basis of urine amount and body postures, such criterion have already been challenged as atypical cases (e.g. horizontal *spraying* with cat in a squatting posture) have been reported with some frequency (LANDSBERG, HUNTHAUSEN; ACKERMAN, 1997; HORWITZ, 2002; BOWEN; HEATH, 2005; PACHEL, 2011). Thus, the concept of normality and abnormality regarding feline elimination patterns deserves more careful investigation.

Cats presenting with inappropriate urination, either in a form of *spraying* or inappropriate *toileting* behaviour (i.e. urine deposited outside the litterbox for the purpose of emptying the bladder), are commonly described as “dirty”, or spiteful, suggesting that they may have the intention to upset the owner (especially in cases in which the problem is displayed in the owner’s absence, seemingly as a projection of the owner’s guilt for leaving the animal alone). When owners are guided about how natural the behaviour is and the underlying motivations of a cat that soils the house, they are likely to confront the problem, and consequently the solution, in a more understanding manner, increasing the chances of a successful outcome. Clear explanations given to the owners constitute a crucial part of the behavioural counseling (LANDSBERG, HUNTHAUSEN, ACKERMAN, 1997), and a key point concerning feline inappropriate urination is to explain the normality of the situation from a feline perspective, bearing in mind, however, the concept of “normality” in this case is prone to changes thus clinicians should keep their knowledge up to date.

1.3 Tinbergen’s four questions and feline inappropriate urination

Feline inappropriate urination both in a form of *spraying* and inappropriate *toileting* behaviour can be interpreted using an integrative scheme: the so called TINBERGEN’S four questions (1963), which seek to understand behaviour in terms

of proximate and ultimate causes along the following four categories: ontogeny, phylogeny, functioning and adaptation. A definition for each of these categories followed by examples for both *spraying* and inappropriate *toileting* behaviour mostly based on scientific evidences can be seen in figure 1.

Figure 1. Feline inappropriate urination according to the four questions of TINBERGEN (1963): ontogeny, functioning, phylogeny and adaptation

ONTOGENY	
DEFINITION	Behaviour is explained on the basis of developmental elements acting within the life time of an individual (Proximate cause).
SPRAYING	INAPPROPRIATE TOILETING BEHAVIOUR
<ul style="list-style-type: none"> - Fearful, anxious, and unpleasant experiences within social relationships (e.g. introduction of a new cat in the group, arrival of a baby etc.) or arisen from poor latrine conditions may lead to <i>spraying</i> which probably acts as a reassuring behaviour (HORWITZ, 2002; BOWEN; HEATH, 2005); - Learning by observation of another “sprayer” cat (anecdotal data); - Organic system functioning under abnormal conditions (e.g. interstitial cystitis) can trigger <i>spraying</i> by generating pain thus leading cat to other potentially less painful urinating postures. The more comfortable feeling thus perpetuates the behaviour by operant conditioning (WESTROPP; BUFFINGTON, 2004). 	<ul style="list-style-type: none"> - Fearful, anxious, and unpleasant experiences around the latrine area (e.g. punishment, threat by another cat etc.): the worse the cat feels in the latrine the less likely to use it; - Individual preferences – the better the cat feels urinating in certain surfaces (e.g. carpet) the more frequent the act (HORWITZ, 2002; BOWEN; HEATH, 2005); - Impaired ability to learn the proper latrine behaviour probably arising from breeding selection in Persian cats (BEAVER, 2005); - Organic system functioning under abnormal conditions (e.g. orthopedic disease) can trigger inappropriate <i>toileting</i> behaviour by generating pain thus leading cat to other locations which may be associated with a less painful movement; the more comfortable feeling thus perpetuates the behaviour by operant conditioning (HORWITZ, 2002; BOWEN; HEATH, 2005).

FUNCTIONING

DEFINITION	
	Behaviour is explained on the basis of how the organic structures of an individual work (Proximate cause).
<i>SPRAYING</i>	<i>INAPPROPRIATE TOILETING BEHAVIOUR</i>
<ul style="list-style-type: none"> - Sex hormones control <i>spraying</i> in entire cats (HART; COOPER, 1984); - Abnormal release of sex hormones can lead to <i>spraying</i> in neutered cats (e.g. in case of adrenal tumor – MILLARD, PICKENS; WELLS, 2009); - Impaired impulse control, anxiety, fear, distress are likely underlying mechanisms of <i>spraying</i> in neutered cats; this hypotheses is based on success achieved by the use of psychotropic agents – for a review, see MILLS, REDGATE; LANDSBERG, 2011); 	<ul style="list-style-type: none"> - Urinary system functioning with polyuria, polacyuria and\or dysuria may lead to inappropriate <i>toieting</i> behaviour by determining urgent urination (e.g. lithyasis, cystitis) (HORWITZ, 2002; BOWEN; HEATH, 2005);

PHYLOGENY

DEFINITION	
	Behaviour is explained on the basis of evolutionary expiation, being unrevealed by reconstructing the history of the <i>species</i> (Ultimate cause).
<i>SPRAYING</i>	<i>INAPPROPRIATE TOILETING BEHAVIOUR</i>
<ul style="list-style-type: none"> - As other felids, cat's ancestor, a small wild African cat, <i>Felis silvestris lybica</i> (DRISCOLL et al., 2007), also exhibits <i>spraying</i> (STUART, 1977); 	<ul style="list-style-type: none"> - As other felids, cat's ancestor, a small wild African cat, <i>Felis silvestris lybica</i> (DRISCOLL et al., 2007), also exhibit scratching-burying urination in several communal latrine areas outside the core territory (STUART, 1977) – in domestic cats such trait may be manifested as a search for more than one place for urination thus leading to inappropriate <i>toieting</i> when latrine spots are in limited number;

ADAPTATION

DEFINITION	Behaviour is explained in terms of natural selection: having solved reproductive or survival problems in the ancestor environment, it is then kept in the descendent individuals (Ultimate cause).
<i>SPRAYING</i>	<i>INAPPROPRIATE TOILETING BEHAVIOUR</i>
- Urine <i>spraying</i> signals different messages such as reproductive status and individual identity (NATOLI, 1989; FELDMAN, 1994), which have helped preventing overt confrontation, and partner selection, both favoring ancestor's group preservation.	- Scratching-burying pattern of urination in several latrine places away from the core territory have made a way in hiding the ancestor's group from predators as well as preventing contamination and spread of diseases (FELDMAN, 1994).

A full range of proximate and ultimate causes in a form of intern motivators and external triggers interplay in determining feline inappropriate urination as either *spraying* or inappropriate *toileting* behaviour. Several aspects, however, still remain to be investigated. For instance, in spite of being a common assumption, the role of *spraying* as bringing reassurance to distressed animals remains to be demonstrated; evidences of anxiety, distress or uncontrolled impulse in these cats are lacking (FRANK, ERB; HOUP, 1999). Furthermore, regarding domestic cats, communication of social status via urine *spraying* is another assumption in need of scientific evidences (NATOLI; DE VITO, 1991). As to inappropriate *toileting* behaviour, contribution of other, harder to diagnose diseases (e.g. interstitial cystitis) requires an in-depth medical evaluation of soiling cats.

In order to control the problem, whilst some of the above mentioned causes can be removed (e.g. medical problems) others are fixed to the environment (e.g. several cats sharing the same space). The solution is therefore helping the cat to accept them or to modeling its response by reacting with other more appropriate behaviours (e.g. whenever feeling distressed the cat may go hide in secure areas rather than performing *spraying* behaviour) (HOWITZ, 2002; BOWEN; HEATH, 2005; HORWITZ; NEILSON, 2007). It is then within the clinical workset and in conjunction

owner-behaviourist that all these will be discussed and the appropriate therapeutics carefully formulated on the basis of the individual cat, owner and household environment (HERRON, 2010).

1.4 Feline inappropriate urination: clinical and social relevance

Inappropriate elimination in a form of *spraying* or inappropriate *toileting* behaviour is the most common problem seen by veterinary experts dealing with feline behavioural cases worldwide. According to Heath (2001), it comprises 35% of the caseloads from German experts, 32% from French and 35% from English. The APBC annual report revealed a prevalence of 39% in a total 65 feline cases recorded from European experts (APBC, 2005). Data from the same institution including 249 feline behavioural cases reported by European experts during 2004 and 2005, which considered both primary and secondary complaints by the owners, showed that 62% of cases involved some form of inappropriate elimination by the cats. In Brazil, the situation appears not to be different - behavioural caseloads from Ramos (unpublished data) pointed out that among 38 feline cases, 37% involved inappropriate elimination (i.e. here inappropriate *toileting* behaviour and *spraying* considered together).

The aforementioned figures are based, however, on the cases seen by behaviour experts; it is also important to understand how the problem presents in the feline population itself as many owners do not seek veterinary for help. There is no available data regarding inappropriate elimination in the feline population in Brazil, but research from other countries such as the United States shows that about 11.5% of cat owners complain about their cats' overall inappropriate elimination (VOITH, 1985). According to Beaver (1989) and MILLS (verbal information)¹, 10% of all cats, whether neutered or not, females and males, will exhibit urine marking as adults.

¹ Verbal information provided by Prof. Daniel S Mills in January/2012.

Apart from its frequent occurrence, inappropriate elimination poses an important social concern: behavioural problems are an important factor when one is deciding to relinquish a cat (SALMAN et al., 2000) and this is the main behavioural reason reported by owners for giving up their cats to a shelter – being mentioned by 43.2% of 1286 cat owners who relinquished their cats (SALMAN et al., 2000). Furthermore, it is a common cause leading to the return to a shelter of a previously adopted cat (CASEY et al., 2009).

Inappropriate elimination is a complex problem which, if left untreated, may result in a break in the cat-owner bond, abandonment or even euthanasia of the cat. Although, recent published caseload series suggest that feline cases of inappropriate elimination are on decline (BAMBERGER et al., 2006; AMAT et al., 2009), this may reflect a move away from specialist treatment. It may be that the general practitioner is better equipped to deal with such cases and/or better quality advice is available to owners.

Veterinary behavioural guidelines commonly propose diagnostic procedures and treatment protocols, for both inappropriate *toileting* behaviour and *spraying*, which are generally clear and reasonably easy to apply, but eradication of the problem is not as simple to achieve (MILLS, REDGATE; LANDSBERG, 2011). Marder and Engel (2002), in a review of 58 cases of *toileting* cats whose owners followed a treatment plan recommended and supervised by specialists, reported that 47% considered the cat to be cured, 26% felt the problem was much better, 14% slightly better, 10% with no change and 3% declared their cats to be worse. Thus, at least 27% of owners were, to a certain degree, unsatisfied; in other words, their cats were still exhibiting the problem at an unacceptable degree.

Higher success rates are generally achieved with *spraying* cases, though (MILLS, REDGATE; LANDSBERG, 2011). In one study, a significant improvement (i.e. 75% reduction of *spraying* behaviour in four weeks) occurred in 80% of the cats (LANDSBERG; WILSON, 2005), which received antidepressant medication. With the use of pheromonatherapy, up to 97% of the cases reached success (PAGEAT, 1996; MILLS; WHITE, 2000; HUNTHAUSEN, 2000; MILLS; MILLS, 2001). However, it is ought to be considered that in some countries such products are either unavailable or

not easily accessed by the owners due to their high prices, thus many cases still remain unsolved; available alternative approaches or behaviour modification on its own does not seem to be as successful, although empirical investigation on this matter is lacking.

Although success rates may seem high, there is clearly a proportion of refractory cases in which typical management regimes do not work. An important question to answer is why this is the case, if we have addressed the cause of the problem. Cases likely to resist to behavioural treatment include those with medical complications in which routinely recommended clinical examinations based on common exams, such as urinalysis and ultrasound of the urinary system may not be sufficient to detect the underlying physical component (CHEW; BUFFINGTON, 2000). Medical check-up is often suggested but is unclear how often this is followed. Also, uncontrollable underlying emotions that may be revealed by behavioural changes and/or changes in neurochemical profile seems relevant for the perpetuation of the problem in other cases.

Recognizing individual differences (MENDL; HARCOUT, 2000) is an integral part of veterinary behavioural medicine, and general rules or characteristics that define individuals can be used to understand them. For example, some cats refuse to defecate and urinate in the same litterbox, some prefer empty trays, a specific substrate or only accept using litterboxes scrupulously clean (NEILSON, 2004 a). Others may manifest the aforementioned atypical forms of inappropriate urination (e.g. horizontal spraying). The importance of individuality thus also deserves investigation.

1.5 The need for further investigations: the present research

Investigations of several aspects of feline inappropriate urination are still lacking. Despite being such a common feline problem for which most behaviour guidelines provide clear explanations and therapeutic recommendations (e.g. HORWITZ, 2002; HORWITZ; NEILSON, 2007), much of the information available

comes from practical experiences and subjective perceptions rather than empirical studies. Further scientific studies are therefore required.

The present research aimed at using a consistent scientific approach to evaluate some of the common assumptions attributed to the cat with inappropriate urination. The problem was investigated in regard to its physiology, behaviour and medical profile, and including some very interesting aspects such as temperament and social status for which there have not been investigations under the scope of feline inappropriate urination.

The objectives of the present investigation were therefore:

- 1.) To determine the prevalence of medical problems among group-housed cats with inappropriate urination (both in a form of *spraying* and inappropriate *toileting* behaviour) by using a general and an in-depth medical work-up;
- 2.) To measure faecal glucocorticoid metabolites and plasma bioamine levels to explore physiological changes in group-housed cats with inappropriate urination (both in a form of *spraying* and inappropriate *toileting* behaviour);
- 3.) To explore previously underevaluated behavioural aspects of group-housed cats with inappropriate urination (both *spraying* and inappropriate *toileting* behaviour) relating to temperament and social organization;
- 4.) To determine differences relating to the aforementioned medical, physiological and behavioural aspects between *spraying* and inappropriate *toileting* behaviour types of inappropriate urination in group-housed cats.

The following session contains a detailed description of the material and methods used in this research. Thus, Chapters 1 and 2 are in the form of scientific journal manuscripts relating to the data for “sprayers” (cats exhibiting *spraying* type of inappropriate urination) (Chapter 1) and “toileters” (cats exhibiting inappropriate *toileting* behaviour) (Chapter 2), respectively. The final session is an integrated discussion of the results, comparing the two groups (i.e. “sprayers” and “toileters”) and final conclusions.

Material and Methods

2 MATERIAL AND METHODS

2.1 Sequence of events

The research was undertaken according to the following order of events: 1 - *Research advertisement* → 2 - *Visits to the households* → 3 - *First visits to the veterinary hospital: preliminary exams.* → 4 - *Second visits to the veterinary hospital: cystoscopies. Behavioural diagnosis* (5) and data collection concerning other behavioural aspects (6- *Behavioural aspects: personality* → 7 - *Behavioural aspects: sociality*) were conducted during visits to the households. At the first visits to the veterinary hospital, cats were recorded (8 - *Recording of cats*) and blood samples were collected for physiological evaluation (9 - *Physiological aspects: blood bioamines*). Faecal samples from the cats were collected by the owners at their homes (10 - *Physiological aspects: faecal glucocorticoids*) and brought during first visits. Medical examinations were therefore performed during first and second visits to the veterinary hospital. Figure 2 illustrates the dynamics of these events.

Figure 2. Flow chart illustrating the dynamics of the experimental stages in this research

2.2 Research advertisement (*Stage 1*)

This research began in January 2010. An information poster (see Appendix A) was posted at strategic points of the veterinary college of the University of São Paulo (FMVZ/USP) including the veterinary hospital (HOVET - FMVZ/USP), and in several veterinary clinics in São Paulo and neighboring cities. It was also sent by email to a list of students of the veterinary college and posted in cat breeders websites and Internet communities about pet cats. Feline trade and exhibition shows were visited by the doctorate student, taking advantage of these occasions to promote the research among many cat owners and other visitors.

Some reputable national websites with a high visitation rate, focused on the theme "pet" (i.e. Saude Animal, Web Animal, Arca Brasil) were also contacted, and as there was interest from their managers, the poster was made available on these sites. A text prepared by the doctorate student, containing further information about the problem of feline inappropriate urination, was also provided to these websites. Within the University of São Paulo (USP) community, the research was released by the USP-News Agency via its website. Research publicity, as described above, was carried out until June/2011 (18 months in total). Such a long period was necessary in order to recruit a sufficient number of cats that could be submitted for medical evaluation given the drop out rate. Owners contacted the doctorate student by email and/or telephone, reported the problem presented by their cats, and indicated their availability to take part in the study. Thus, a visit to their households was scheduled.

This study was granted ethical approval by the University of São Paulo Veterinary College's Ethics Committee. Participants were contacted after completion of the study and given a brief report of the main results, opportunity to ask questions about any of the results and permission sought to publish the results. Subsequently, all contacted participants consented to publication of the data anonymously.

Financial support was provided by FAPESP (São Paulo state research funding agency).

2.3 Visits to the households (*Stage 2*)

Visits to the households started in March/2010 and were conducted until June/2011. With about one to two visits a day, once or twice a week, cat owners of several regions of Sao Paulo city, who had previously agreed to take their cats to the university veterinary hospital (HOVET- FMVZ\USP) for the medical examinations afterwards, were visited. Some owners living in other areas (i.e. in the countryside cities of Campinas, Jundiai, Amparo and Santo Andre) were visited during weekends. A total of 41 (forty-one) households were visited. About 80% of the owners who made initial contact were visited; the remaining ones contacted the researcher but a visit was not scheduled due to several reasons: cats were intact, cats presenting inappropriate urination also had other symptoms of organic disease, owner was not available neither to receive the visit of the researcher nor to take the cats to the university veterinary hospital afterwards etc.

From each multi-cat household (they contained 3-9 cats), at least a dyad was selected to form a "case-control" study. This consisted of a cat displaying inappropriate urination (i.e. the "case" cat), and another cat, preferably of same sex and age as the "case" cat, described by the owner as never displaying inappropriate urination (i.e. "control" cat), both neutered and apparently healthy. This strategy allowed us to further identify, based on the differences between "cases" and "controls", medical, physiological and behavioral factors related to the individual (i.e. the cat, not the environment in which it lives) potentially associated with inappropriate urination. From the 41 households visited, 55 "case-control" dyads were selected; in a few households, two dyads could be selected.

During visits, the owners were informed about the research objectives as well as its stages. Once the "case-control" dyad was selected, demographic information regarding the cats, the households as well as information about the problem were gathered (see the latter in Appendix B). Furthermore, behavioural diagnosis (Stage 5 – see 2.3.1) was made and behavioural data concerning personality (Stage 6 – see 2.3.2) and sociality (Stage 7 – see 2.3.3) were collected.

Owners were then informed about the process for evaluation of feline stress via faecal glucocorticoid metabolites, which constituted *Stage 10* (see 2.4.3) of this research. They were told about how to proceed with the faecal collections of both cats, "case" and "control", which had to be completed before the medical examinations conducted at the university veterinary hospital. Detailed instructions regarding the individual collection and the immediate storage in the owner's freezer were given to them. Given the difficulties involved in the procedure, considering the existing number of cats in the household and the need to witness the defecation, a specific frequency of collection was not defined. It was stipulated that the collection should be on different days and a maximum period of 30-60 days was available for completion. The owners received 10 collection pots as 5 samples per cat were to be collected. A thermal box and recyclable ice were also provided as they were used for transport of the frozen samples until their arrival at the university.

If the owner agreed with the requirements, a visit to the veterinary hospital was scheduled; confirmation thus depended upon faecal sampling completion.

2.3.1 Behavioural diagnosis (Stage 5)

Following the "key" features proposed by Horwitz (2002) for the behavioural diagnosis of feline inappropriate urination (i.e. urine amount and distribution outside the litterbox, type of surface in which urine is deposited inappropriately, cat's posture during inappropriate urination, and litterbox use), a questionnaire (see Appendix C) was developed. This was completed by the doctorate student during the visits in consultation with the owner. At the end, a conclusion was drawn about the pattern of inappropriate urination displayed by the "case" cat, thus determining to which of the following groups the pair would be allocated (i.e. Group 1: urine *spraying*, Group 2: inappropriate *toileting* behaviour). In case of any ambiguity, a European specialist in veterinary behavioural medicine was consulted. Fifty-five

"case control" dyads were initially selected, with the following Groups composed: Group 1 – 21 dyads, Group 2 – 34 dyads.

Group 1 dyads came from households that might also have cats that failed to use the normal latrine for urination; those cats, however, were not selected for inclusion in the study. All "case" cats in group 1 exhibited vertical *spraying*. A couple of them, according to the owners, also deposited urine horizontally. Since this occurred only occasionally and it was likely to represent a form of horizontal *spraying* (rather than a simple refuse to use the appropriate latrine for urination) (PRYOR et al., 2001; TYNES et al., 2003), these cats were kept in the study.

As to Group 2, in the case of five dyads, their households also had *spraying* cats and were therefore excluded from the study. Thus, all the remaining dyads in Group 2 (n= 29) came from households which did not have any cat exhibiting urine *spraying*.

Cats displaying any other symptoms besides inappropriate urination, or any other overt behavioural problem, were considered to be unhealthy and thus were not selected for inclusion in either of the groups.

Cats that entered the litterbox but urinated with a standing posture thus depositing urine outside were not included in any of the groups as this could be a form of normal urination as opposed to *spraying* behaviour (LANDSBERG, HUNTHAUSEN; ACKERMAN, 1997). In only one of the visited households there were cats exhibiting this pattern of urination and therefore were not selected.

Similarly, cats showing inappropriate urination using a squatting position restricted to some specific valuable items such as the owner's clothes were not selected as this could be a form of horizontal *spraying* or attachment problem (SCHWARTZ, 2002) rather than just a rejection of the appropriate toilet place (BOWEN; HEATH, 2005). None of the cats, considering all the visited households, however, presented this form of inappropriate elimination.

2.3.2 Behavioural aspects: personality (Stage 6)

During the visit to the household, the personality questionnaire (see Appendix D), and detailed guidelines for its completion were given to the owners. The questionnaire was to be answered by the owner and, independently, by another family member who had a reasonable knowledge of the cats. This procedure, suggested by Meagher (2009) for studies based on observers' opinions, aimed at assessing the reliability of the responses given by the owners. A questionnaire regarding the "case" cat, and another one for the "control" cat, were completed by each respondent. The questionnaires were then handed in to the doctorate student at the first visit to the veterinary hospital when cats were brought for the medical examinations.

The questionnaire consisted of 10 questions, with answers given in a graduated scale (i.e. a line containing 10 marks). Thus, respondents answered according to how they believed a characteristic (e.g. *curious*) was representative of the behaviour of a given cat, placing its response from "nothing" to "totally". First, in the black line, owners responded relatively to their cat, "case," and next, in the red line, in relation to cats in general. An identical questionnaire was then answered for the "control" cat, by the same respondent. Whenever possible, the other family member who had close contact with cats did the same, thus four questionnaires were collected per household: two about the "case" cat and two for the "control" cat.

The questionnaire was adapted from the constructs identified by Feaver, Mendl and Bateson (1986), containing 7 of the behavioral characteristics used in the original work (i.e. *active, curious, sociable with people, fearful of people, hostile to people, tense, equable with cats*), accompanied by their definitions. Three final items about behavioral styles (i.e. personalities: "bossy," "timid," "easy-going") were also included - these were answered only in relation to the participant cats ("case" and "control") and not about cats in general.

These final three questions served a reliability process as the scores by the owner and other family member were compared, as suggested by Meagher (2009).

For the questionnaire analysis, the following procedures were attended:

1 - For all questionnaires, markings on the graduated scales were initially transformed into scores, which corresponded to the numerical grade from the scale.

Intermediate marks, whatever, were always approximated to 0.5, as shown in figure 3.

Figure 3. Scoring system. One of the seven behavioural characteristics (active), as it was presented at the personality questionnaire

2 - A comparison was then made between the scores for "cats in general" (red line) given by the owners in the two questionnaires (i.e. relating to "case" and "control"). Since in both questionnaires, owners should have responded with their own idea of how a given characteristic was represented in cats in general, it was expected that there should be similar scores for "cats in general", in the two questionnaires, for all seven behavioral characteristics. This allowed reliability assessment (MEAGHER, 2009).

3 - From the scores given by the owners for each of the seven behavioural characteristics, for both "case" and "control" cats, the scores assigned by them for "cats in general" were subtracted. By doing this, a relative score representing how a certain behavioural characteristic was perceived in "case" and "control" cats was extracted. Importantly, the resulting scores took into account the very subjective notion owners had for each of the behavioural characteristics.

4 - If the answers given by the owners were reliable, the scores resulting from the above mentioned system, for both "case" and "control" cats, were used. "Cases" and "controls" were then compared in terms of average scores for the seven behavioural characteristics as well as the three personalities (i.e. "bossy", "timid", "easy-going").

2.3.3. Behavioural aspects: sociality (Stage 7)

For the evaluation of social organization and status, a short questionnaire (see Appendix E), asked the owners about how "dominant" and "submissive", within the multi-cat group, they viewed their "case" and "control" cats. These were answered during the visits to the households. As in the previous stage, the answers were provided through a graduated scale (i.e. a line containing 10 marks). Their answers were based on how they believed these characteristics (e.g. "dominant," "submissive") were representative of the behavior of their cats, locating their response on the scale from "nothing" to "totally". Furthermore, social matrices (see Appendix F), composed of feline affiliative (*sleeping together, licking each other, rubbing against each other*) and agonistic (*aggression*) behaviours, were completed by the researcher as the owner described the observed interactions between their cats. The affiliative and agonistic interactions contained in the social matrices were described by the owners according to their direction (i.e. who starts/gives and who receives) and their frequency of occurrence (*almost always, often, occasionally, rarely, almost never*).

Evaluation of affiliative interactions within the multi-cat groups allowed identification of social organization. If affiliation existed one could say the cats were integrated socially as opposed to living independently within the same environment. On the other hand, a close look at agonistic networks permitted identification of dominant and submissive relationships between cohabiting cats. As an initial analysis step, social interactions gathered in the social matrices were described visually individually for each of the households. Graphical social network representations, with cats identified, together with the frequency and direction of the social interactions, were made for each of the households for each of the types of interaction. An example of such representation can be seen in figure 4.

Figure 4. Graphical representation of social interaction (agonism) derived from the social matrices in one of the households

Legend	
<div style="border: 1px solid black; width: 20px; height: 10px; display: inline-block;"></div>	Animal Case
<div style="border: 1px dashed black; width: 20px; height: 10px; display: inline-block;"></div>	Animal Control
	Almost Never
<div style="width: 20px; height: 5px; background: linear-gradient(to right, yellow, red); display: inline-block;"></div>	Rarely
<div style="width: 20px; height: 5px; background: linear-gradient(to right, red, blue); display: inline-block;"></div>	Occasionally
<div style="width: 20px; height: 5px; background: linear-gradient(to right, blue, green); display: inline-block;"></div>	Often
<div style="width: 20px; height: 5px; background: linear-gradient(to right, green, black); display: inline-block;"></div>	Almost Always

A sociometric method was then applied. For this, all frequencies of affiliative interactions (*sleeping together, licking each other, rubbing against each other*, considered all together) were firstly converted into scores (i.e. almost always =4, frequently=3, occasionally=2; rarely=1, almost never=0), and on the basis of these, *Jaccard* indexes were calculated with the *Simejac* software as described by the *Dominance Directed Tree*

Method (IZAR; SATO, 1997; IZAR, JAPYASSÚ; ALBERTS, 2005). This led to *Affiliative trees* which allowed identification of the strongest associations considering all the cats in the multi-cat houses. More associated individuals were linked by shorter lines within the “trees”. To evaluate whether the associations between “case” cats and other members of the group differed from the ones between “controls” and others, frequency of affiliative interactions were statistically compared (i.e. frequency of “cases” - others versus “controls”-others).

Agonistic interactions were also investigated by the sociometric *Dominance Directed Tree Method* (IZAR; SATO, 1997; IZAR, JAPYASSÚ; ALBERTS, 2005) For this, all frequencies of agonistic interactions (i.e. aggression) were firstly converted into scores (i.e. almost always =4, frequently=3, occasionally=2; rarely=1, almost never=0), and on the basis of these, and using the *Domina* software, *Dominance trees* were constructed (IZAR; SATO, 1997; IZAR, JAPYASSÚ, ALBERTS, 2005). It was thus possible to indentify the dominance indexes (i.e. *tree rank* and *number of individuals which it dominates*) of each of the individuals, which were calculated on the basis of how frequently the cat initiated agonistic interactions. In order to evaluate whether “case” cats differed from “control” cats, these were compared in terms of frequency of agonistic interaction in which the cat was involved. Furthermore, a comparison was made between “case” and “control” cats as a function of *tree rank* and *number of dominated individuals*.

Regarding the questions about "dominance" and "submissiveness", as with the personality questionnaire, markings on the graduated scales were initially transformed into scores, which corresponded to the numerical grade from the scale. Intermediate marks were always approximated to 0.5, as shown in figure 5. A score for dominance and submissiveness, for each of the pairs of cats, “cases” and “controls”, was then determined. Subtracting the submission scores from the dominance scores led to the *dominance index*. “Cases” and “controls” were then compared as a function of the above three variables.

Figure 5. Scoring system. Questions on *dominance* and *submissiveness* as it was presented at the social questionnaire

2.4 First visits to the veterinary hospital (*Stage 3*)

When the owner had completed faecal sampling, or had reached the maximum period established for the collection, the first visit to the veterinary hospital was scheduled. This involved the following preliminary clinical examinations: complete blood count, serum biochemistry (renal and hepatic profiles), urinalysis, urine culture and ultrasound of the urinary system. The aim was to identify cats suffering from any medical condition, since this research focuses on inappropriate urination of exclusive behavioural causes. Thus, although all cats presented as healthy at the time of recruitment, only those that were shown to be free of physical illness in these exams were included in the final analysis. Any abnormality of the lower urinary tract (e.g. bladder urolithiasis, cystitis) or alterations indicative of either a systemic disease or a dysfunctional organ led to exclusion of the cat and, therefore, of the dyad. Alterations of the upper urinary tract which, however, did not show to interfere with

functioning of the urinary system (e.g. renal urolithiasis accompanied by normal urinalysis and renal profile) did not lead to cat's exclusion.

All the procedures involved in these exams were conducted with both cats from the household, "case" and "control", on the same occasion, in the veterinary hospital (HOVET-USP), in a room dedicated to this research, during the morning. The collected materials were processed according to standards and techniques established by the Laboratory of Clinical Pathology (HOVET-USP). As for the ultrasounds, they were performed in the same room, with mobile equipment owned by a veterinarian member of the research team (Mariana Freitas), specialist in veterinary ultrasonography, and who carried out all the examinations.

In preparation for the preliminary examinations, the owners were told to fast cats for at least 8 hours (maximum of twelve hours). In the last two hours preceding the exams, owners were instructed to encourage water intake, and to prevent the use of the litterbox in an attempt to promote bladder filling, necessary for the collection of urine, which was done by cystocentesis guided by ultrasound. The aim was to collect a sufficient amount of urine (approximately 8-10 mL) in order to conduct both urinalysis and urine culture; if this was not possible, the dyad was excluded from the study since a complete medical check-up could not be performed.

During visits to the veterinary hospital, besides these preliminary examinations, the behaviour of cats was video recorded (Stage 8 - see 2.4.1). Furthermore, blood collection was performed for physiological evaluation (Stage 9, see 2.4.2). Blood samples had to serve for at least complete blood count and biochemistry profile in order to decide whether the dyad could be recruited. Whenever blood collection was not conducted, or the amount was insufficient, the dyad was excluded from the study since exams could not be completely performed. If blood was enough for the exams but not for physiological evaluation (Stage 9), the latter was not performed but the dyad was kept in the study if exams resulted normal. Ideally, 6 ml of blood were enough for serving both the preliminary examinations and physiological evaluation.

Faecal samples were brought by the owners during this visit and allowed further physiological evaluation (Stage 10, see 2.4.3). If collection was not made, or the

established number of samples was not achieved, the dyad was retained in the study if the preliminary examinations were performed and resulted normal.

The above mentioned exams were performed exclusively to rule out medical conditions among the recruited cats. However, serum creatinine, serum urea and leucocytes count were also used in order to compare “case” and “control” cats.

2.4.1 Recording of cats behaviour (Stage 8)

In order to evaluate the behaviour exhibited by the participant cats, they were all video recorded during their first visit to the veterinary hospital, in the same room where the medical examinations were performed. The owner arrived at the veterinary hospital at a pre-scheduled time, always in the morning, and a sequence of activities was carried out, in the same order (see figure 6).

Two video recording sessions were conducted independently for each of the cats. Only owner and cat were present in the room during video sessions, which lasted five minutes. In the first session, the owner was instructed to place the cat on the floor as soon as the doctorate student left the room. This was the moment the recording began. The cat carrier was kept closed, placed in a designated location within the recording area. The cat was then left free in the room for about three minutes. The owner was instructed to remain seated in the designated chair the whole time, without promoting any interaction with the cat.

After the first three minutes of recording, the student entered the room, having knocked on the door, and put a saucer containing feline wet food on the floor, near the chair where the owner was seated. The student then left the room, and the second session began when the owner placed the cat on the floor in front of the saucer. The same recommendations were given and the cat remained free in the room for another two minutes. At this point, the student entered the room, announcing the end of the second recording session.

In a pre-defined randomization schedule, "cases" and "controls" were alternated as to who was the first to be recorded. Behavioural categories and their defined behaviours (see figure 7), were defined before video analysis started. The videos were then evaluated methodically by a "blind" observer who did not know any of the cats and thus was not influenced by any previous knowledge of the recorded subjects. This observer was a biologist who owned cats herself.

A Sony Camcorder 19TRV was used for video recording. Digitalization was then made by Windows Movie Maker 2.6 and coding by the software Solomon Coder beta 11.04.23 (ELTE TTK, Department of Ethology, Budapest).

Figure 6. Flow chart illustrating the dynamics of events during the first visit to the veterinary hospital, which could last about one to two hours

Figure 7. Behavioural categories and defined behaviours for video analysis

BEHAVIOURAL CATEGORY 1 - LOCATION

*ZONE 1 – left top square – where owner’s chair is located –A**

*ZONE 2 – right top square - where cat’s carrier is located –B**

*ZONE 3 – left bottom square – where counter is located – C**

*ZONE 4 – right bottom square – where examination table is located –D**

OUT OF VIEW – if on the floor next to the tripod with the camera

HIDEN PLACE– under the medical wardrobe (this was blocked by a cardboard plaque thus to access it cats had to work for moving the plaque)

*UNDER THE CHAIR – in zone 1, under the chair where owner remains seated – E**

*OWNER’S LAP – in zone 1, up the chair on the owners lap – F**

*COUNTER – in zone 3, on the top of the counter – G**

*EXAMINATION TABLE – in zone 4, on the top of the examination table – H**

*CARRIER – in zone 2, on the top of the cat’s carrier – I**

**For a better understanding of the above mentioned locations see figure 8.*

BEHAVIOURAL CATEGORY 2: BODY POSTURE

SIT - cat is positioned with its front legs extended straight, front paws and rump on the ground

LYE DOWN – cat is positioned with four legs and abdomen on the ground

STAY – cat is positioned with four legs extended straight, stationary

STAY COWERED – cat is positioned with four paws on the ground and abdomen near the ground

MOVE – cat walks or runs in the room

MOVE FEARFULLY – cat walks or runs in the room with abdomen near the ground

BEHAVIOURAL CATEGORY 3: ACTIVITY

LOOK AT THE OWNER – cat directs the head, and maybe the neck, whilst standing still, with eyes focused on the owner

INVESTIGATE THE CARRIER – cat gets close by sniffing, pawing, rubbing, or scratching the carrier

INVESTIGATE THE OWNER – cat gets close by sniffing, pawing, rubbing, or scratching the owner

EAT FOOD – cat ingests the food offered

CATEGORY 4: VOCALIZATION

MEOW – cat vocalizes by meowing

Figure 8. Examination room where recording of cats took place. Locations are identified by letters A-I and described in figure 7. *HIDDEN PLACE: from a window at the door it was possible to see the moment the cat went from out of view to the hidden place

"Case" and "control" cats were compared as a function of duration on the following behaviours: at the hidden place, close to the owner (zone 1 + zone 2 + under the owners' chair), far from the owner (zone 3 + zone 4 + out of view), stopped (sit + lie down + stay + stay cowered), moving, attentive to the owner (looking owner + investigating owner). "*Far from owner/ (far+close) from owner*" and "*Moving/ (stopped+moving)*" proportions were calculated and then used for a comparison between "cases" and "controls". Comparisons were also made between "cases" and "controls" as a function of frequency of the following: meow, attentive to the owner. Other behavioural variables were described separately for "cases" and "controls".

2.4.2 Physiological aspects: blood bioamines (Stage 9)

A collection of approximately 6 ml of blood from the jugular vein of each of the cats, "case" and "control", was performed at the end of the first visit to the veterinary hospital, as shown in figure 6. Part of this material was used to perform complete blood count and serum biochemistry. The remaining (approximately 4 ml), transferred to EDTA tubes, was used for determining blood bioamines. These, in conjunction with faecal glucocorticoids (See 2.4.3, Stage 10), served as physiological parameters for the assessment of stress and emotionality in the participant cats.

Given some bioamines' photosensitivity, EDTA tubes were protected from light by aluminum foil. Within about 15 (fifteen) minutes after collection, the tubes were centrifuged for 15 minutes under refrigeration (i.e. 4C), at 3,000rpm. The supernatant was then transferred in duplicate amber eppendorfs (approximately 1 mL in each). The eppendorfs were then stored in a freezer (-80C) until dosages; these were carried out at once, in May-June 2011.

Bioamines dosages were performed in the Department of Nephrology (Sao Paulo Federal University, UNIFESP), under coordination of Prof. Dulce Casarini. The doctorate student had the chance to follow extraction procedures which prepared the samples to undergo chromatography; high performance liquid chromatography

(HPLC) was coupled to an electrochemical detector. Extraction and quantification were undertaken according to procedures used routinely by the aforementioned department. Such method has previously proved to be both valid and reliable (NAFFAH-MAZZACORATTI et al., 1990; 1992). Blood concentrations of serotonin, dopamine, norepinephrine and epinephrine were measured.

The following two reliability tests were also performed:

1 - *Same samples stored in both EDTA and heparin tubes (for measuring catecholamines) as well as in EDTA and dry tubes (for measuring serotonin):* blood samples from seven cats of the university shelter were placed in three types of tubes - EDTA, heparin and dry. Catecholamines concentrations were determined from extracts derived from EDTA and heparin tubes. In the same way, serotonin concentration was determined from extracts derived from EDTA and dry tubes. As in human medicine heparin and dry tubes are elected for catecholamine and serotonin dosages (NAFFAH-MAZZACORATTI et al., 1990; 1992), respectively, their resulting dosages were taken as references. Thus, in order to check for reliability, EDTA concentrations were compared to heparin and dry concentrations;

2 - *Samples from the same cats stored in EDTA tubes then placed in duplicate eppendorfs and analyzed independently (measurement of catecholamines and serotonin):* samples collected from five cats owned by members of the research team were stored in EDTA tubes then transferred to duplicate eppendorf tubes and labeled as different cats. In order to test for reliability, concentrations from these duplicates were compared.

For the reliability tests, correlations between measurements taken from different methods were calculated. If values derived from samples stored in EDTA tubes proved to be reliable, comparisons were then made between “cases” and “controls” for serotonin, norepinephrine, epinephrine and dopamine.

2.4.3 Physiological aspects: faecal glucocorticoids (Stage 10)

Ten samples (five of the "case" cat and five of the "control" cat), were brought by the owners during first visit to the veterinary hospital. Samples were kept at the university freezer (-80C) and thus transferred to polypropylene tubes and stored in a -80C freezer once selection of the dyads was confirmed through the preliminary examinations

In May 2011, samples were dried and weighed, and glucocorticoids were extracted and dried at the Sao Paulo State University (UNESP, Jaboticabal). For these, 0.2 g aliquots of dry faeces were placed in vials of glass (15 mL) to which 5 mL methanol (90% methanol: 10% pure water) were added. A multi vortex unit was used for a 15 minute homogenization of the sample followed by 15 minutes 3,000 rpm centrifugation. Extracts were then dried for transportation, all at once, in eppendorf tubes. In July 2011, at the Vienna School of Veterinary Medicine, faecal analyses were conducted, under supervision of Prof. Rupert Palme.

Re-suspension and dilution procedures were undertaken according to the methods used in the aforementioned institution (PALME et al., 2001, SCHATZ; PALME, 2001). Samples were then analyzed by 11,17 dioxoandrostanes enzyme immunoassay (EIA). This method has proved to be both valid and reliable, being tested in domestic cats in at least two studies by the same research team that conducted analysis (PALME et al., 2001, SCHATZ; PALME, 2001). A group of glucocorticoid metabolites highly excreted by domestic cats, 11-oxoaetiocholanolone, was measured.

For each cat it was considered a median of the four dosages related to the four faecal samples. Faecal glucocorticoid metabolites medians values were then used for a comparison between "case" and "control" cats.

2.5 Second visits to the veterinary hospital (Stage 4)

Second visits to the veterinary hospital were planned for cystoscopies of suitable female cats. Once preliminary examinations had revealed normal results, a

given dyad was considered selected, and a second visit was then scheduled. A specific week day was selected as the entire room (also used for exclusionary exams and recordings) needed to be re-arranged to accommodate all the cystoscopic and anesthesia equipment.

A period of training during which the cystoscopies were performed with cat cadavers and cats from the university cattery, preceded the execution of the exams in participant cats. From September 2010 on, cystoscopies were carried out in selected female “case” cats (i.e. those exhibiting inappropriate urination) of both groups. Since these cats did not show any abnormality in the preliminary exams and so were part of the study, cystoscopy allowed us to investigate abnormalities that may have passed unnoticed even by complementary exams (e.g. interstitial cystitis) (CHEW; BUFFINGTON, 2000).

Out of the 23 recruited "case-control" dyads (i.e. after exclusion of unhealthy dyads given the results of preliminary exams), 12 (2 from Group 1 and 10 from Group 2) included female “case” cats, suitable for cystoscopic examination. The procedure was described to the owners by the doctorate student, so they could then decide whether or not to allow their cats to be submitted for examination. The need for anesthesia and its risks were clearly explained to them. Eight owners decided in favor of the examination.

Cystoscopies were performed by the supervisor of this research (Prof. Archivaldo Reche Junior) with the assistance of a veterinary member of the research team (MSc Alexandre Daniel), and a veterinary anesthetist (Geni Patricio) from the Department of Animal Surgery (VCI-FMVZ/USP). In preparation for the exam, the owners were told to fast the animal for at least 8 hours (maximum of twelve hours) and to take away water two hours before the exam. Cats were pretreated with acepromazine and meperidine (0.05mg/kg and 4mg/kg, respectively, IM). Anesthesia was then induced with propofol (5-6 mg/kg, IV) and maintained with isoflurane diluted in 100% oxygen at concentrations necessary to maintain the third level of the third stage of Guedel’s classification of depth of anesthesia (1937). In the case of three females which showed to be aggressive, ketamine (2 mg/kg IV) was added to the premedication, which allowed us to safely handle them whilst

promoting induction with propofol. In the last four procedures, in order to achieve a better penetration of the endoscope via a deeper urethral relaxation, butorphanol (0.4 mg/kg IV) was added to the protocol.

The following are some pictures of the cystoscopic equipment, in use during one of the cystoscopies:

Figure 9. A - Cystoscopic equipment; B - Cystoscopy being performed.

2.6 Data Analysis

For the statistical process, Shapiro-Wilk normality test was first performed for each of the aforementioned variables in both groups (group 1: urine *spraying*, group 2: inappropriate *toileting* behaviour; “cases” and “controls” were tested in separate). In case of normal distribution for both “cases” and “controls”, Student paired t-test was selected for a comparison between them in that given group. Wilcoxon signed-rank test was chosen in case of not normal distribution in the tests (i.e. “cases”, “controls”, or both of them). As a complementary phase, groups 1 and 2 were compared considering their participant cats altogether (i.e. “cases” + “controls”). For this, Two sample t-test or Kruskal-Wallis test was used depending on the distribution of the variable: if a non normal distribution was previously seen in at least one test (“cases”, “controls”, or both of them) in one of the groups, a non-parametric test was used. Statistical analysis was performed using SAS software (9.2 version) and a probability level of 0.05 was used to assess significance.

For the reliability assessments, Intra-class correlation test (FLEISS, 1981) was used with the SPSS software (version 19.0 for Windows).

2.7 Selection of Participants – Experimental Groups 1 and 2

2.7.1 Group 1 (“Sprayers”)

Twenty-one urine *spraying* “case-control” dyads (i.e. Group 1) were initially selected. They were then scheduled for the preliminary exams, which were conducted at the university veterinary hospital (HOVET-USP), with a view to selection of participant cats.

In the case of one of these dyads, a second urine collection was recommended, this time conducted by the owner at home and via spontaneous urination. This was

proposed because changes were found in the urinalysis of the “case” cat (i.e. presence of hemoglobin and increased amount of red blood cells) and these could be due to the cystocentesis procedure since the rest of the examination did not reveal abnormalities. The owner, however, refused to perform such a collection, and did not agree to bring the cat to the veterinary hospital for a second urine collection, therefore a complete medical examination of this dyad was not possible. The dyad was then excluded from the research.

In the case of another two “case-control” pairs, sometime after the preliminary exams had been performed, the owners contacted the doctorate student to report that, contrary to what they had thought, they noticed the “case” cat using the litter box in an unusual way. The cat had entered the litter box but assumed a standing posture for urination as if *spraying*. The behaviour could denote urine marking, but could also be just an unusual posture of normal urination. Due to this, both cats were excluded from the study.

Complete preliminary exams were achieved in 18 urine *spraying* “case-control” dyads. As part of these dyads presented medical problems in both or one of the cats, we ended up with 11 “case-control” *spraying* dyads (see figure 10).

Eleven urine *spraying* “case-control” dyads were therefore recruited to Group 1. Among the 11 “case” cats composing Group 1, there were two females and nine males; ten mixed-breeds and one Persian. Their average age was 6.27 years old (SD=2.44). As for the “control” cats, they were 6 females and 5 males; 10 of mixed-breeds and one Siamese. Their average age was 6.43 years old (SD=4.48). Households recruited for Group 1 had on average 6.09 cats. Four of the 11 (eleven) “case-control” dyads came from two households; there were therefore 9 (nine) different households in Group 1.

Figure 10. Flow chart of the recruitment and selection process for cats in Group 1 (“sprayers”)

GROUP 1: URINE SPRAYING

PRELIMINARY EXAMS OF THE 18 REMAINING DYADS:

✚ 11 HEALTHY “SPRAYERS”: *speedy-cadu/ gorda-quick/ marcel-luci/ oscarito-lilea/ mussum-mi/ gutu-thiago/ med-foguinho/ nino-pitulo/ fofa-cotoca/ mitchu-et/ ronin-macaca/*

✚ 7 MEDICAL “SPRAYERS”:

-only “case” cat with medical problem: *menininha-loirinha/ chico- vênus/ cookie-floquinho/ manolo-poncio/ pacatu-lelis/ salém-tifu*

-both “case” and “control” cats with medical problem: *fofo-picachu*

2.7.2 Group 2 ("Toileters")

For Group 2, preliminary exams were performed in 31 of the 34 "case-control" dyads initially selected. Three pairs were not examined because in one case, the "case" cat, who was sixteen, died a few weeks after the visit to the household; in another, the "case" cat got sick (i.e. hepatic disease) before the examination and in the third case, "case" cat needed to be submitted to perineal uretrostomy due to an obstructive urinary disease; therefore, in the absence of a "control" cat in the same condition in the household the cat was excluded from the study.

Out the 31 "case-control" examined pairs, the results of only 28 were considered in the decision for selection to the study. As to one of the missing dyads, excessive aggression by the "case" cat made blood collection impossible, thus generating incomplete results for the preliminary exams. In a second pair, the owner realized, before bringing the cats to the university, that, in fact, the cat initially selected as "control" also showed inappropriate urination. Finally, in a third pair, a second urine collection was recommended, this time by spontaneous voiding at home. This was because in the urinalysis "case" cat alterations possibly resulting from cystocentesis were seen. Since the owner did not agree to attend to this, the dyad was excluded from the research.

Among the remaining 28 dyads five were from households where other cats exhibited urine *spraying*. All of these, however, as said earlier, had medical problems and thus their *spraying* could be due to non-behavioural reasons. Independently of this, as our goal was to evaluate households with no problems of *spraying*, the five pairs were not considered in the research. Thus, 23 pairs were selected for Group 2, who lived in households without the problem of urine *spraying*. As part of these dyads presented medical problems in both or one of the cats, we ended up with 12 "case-control" inappropriate *toileting* dyads (see figure 11).

Among the 12 "case" cats composing Group 2, there were ten females and two males; six mixed-breeds, two Persians, two Maine Coons and two Persians. Their average age was 4.46 years old (SD=2.19). As for the "control" cats, they

were 7 females and 5 males; 9 of mixed-breeds, 2 Maine Coons and 1 Persian. Their average age was 5.21 years old (SD=3.35). Households recruited for Group 2 had on average 4.58 cats. Two of the 12 “case-control” dyads came from only one household; there were then 11 (eleven) different households in Group 2.

Results obtained from behavioural, medical, and physiological evaluations will be presented only about the above mentioned 23 healthy “case-control” selected dyads, in the course of the following two chapters.

Figure 11. Flow chart of the recruitment process for cats selected for Group 2 (“toileters”)

GROUP 2: INAPPROPRIATE TOILETING BEHAVIOUR

PRELIMINARY EXAMS OF THE 23 REMAINING DYADS:

✚ 12 HEALTHY “TOILETTERS”: *monalisa-bia/ tunico-francesco/ mila-horacio/ natasha-floripa/ ágata-mel/ darah-thor/ brisa-toquinho/ mel-babalu/ marie-kiara/ stephania-pollux/ lua-mila/ diná-cleo*

✚ 11 MEDICAL “TOILETTERS”

-only “case” cat with medical problem *isis-thor/sofia-lara/sassy-meggy/igor-minnie/gasper-tracey*

-both “case” and “control” cats with medical problem: *tides-valentino/ ignácio-bartô/ lino-nestor/kitty-kate*

-only “control” cat with medical problems: *gigi-greg/ pantufa-giedi*

Chapter 1:

"Medical, physiological and behavioural aspects of cats exhibiting *urine spraying*: a case-control study with multi-cat households"

Abstract

Urine *spraying* is a typical form of scent mark by feral cats and a common behavioural problem exhibited by owned cats. Concepts regarding underlying causes of the behaviour (e.g. social “dominance”) have been transported from studies with feral cats even in the absence of scientific data to support it. A study with 18 “case-control” dyads in which the “case” cats presented urine *spraying* was conducted in order to investigate aspects potentially related to the problem, specifically: disease, stress, personality, emotionality, social behaviour. Among the participant “case” cats there were 9 males and 2 females. About thirty-nine percent of “sprayers” (as opposed to 5.6% “controls”) had a medical problem interfering either with the urinary system or with the overall organism functioning. When alterations of the upper urinary system (with no obvious influence on urinary functioning) were considered, figures increased to the following: 66.7% (“sprayers”) versus 16.7% (“controls”) presented medical alterations. No differences between “cases” and “controls” were seen with regard to faecal glucocorticoid metabolites and plasma dopamine, but the former indicated that both “cases” and “controls” were in a high arousal emotional state. They also did not differ in the behaviours recorded and in terms of personality and social behaviour rated by the owners. However, they showed to be unreliable in their opinions towards several behavioural aspects of their cats’ personality. Results suggest the importance of detailed medical check-ups in cases of urine *spraying* by domestic owned cats. *Spraying* behaviour as a possible coping strategy is also discussed.

Key-words: marking, stress, dominance, personality, behaviour

3.1 INTRODUCTION

Scent marking in the form of urine *spraying* is a common behaviour within the felidae family (SMITH et al., 1989; KITCHNER, 1991). From small felines such as ocelots (WELLER; BENETT, 2001) to big cats as lions and leopards (SCHALER, 1972; BOTHMA; LE RICHE, 1995) individuals of both sexes, either as captive or in the wild, exhibit *spraying* as a normal part of their behaviour repertoire. Some variation is seen among felid species (e.g. whilst female Kalahari leopards are hardly ever seen scent marking by *spraying*, female lions are reported to scent mark more frequently than male counterparts – SCHALER, 1972; BOTHMA; LE RICHE, 1995), the main purposes of *spraying* in wild cats, as proposed by Smith et al. (1989) encompass establishment and maintenance of territory boundaries and advertisement of female reproductive status.

Urine *spraying* is the predominant method of scent marking exhibited by domestic feral cats (FELDMAN, 1994). It is mostly performed by adult males while travelling along well-defined communally used paths of their range (LEYHAUSEN, 1979; LIBERG, 1980; FELDMAN, 1994). According to Dehasse (1997), *spraying* is triggered in the following five situations: excitement, communication, perturbation of familiar marking, presence of an activating mark and presence of an activating spot. However, characteristics and functions of urine marking in domestic cats vary as a function of their spacing patterns (CORBETT, 1979). Domestic cats differ in their spatial and thus social organization depending on the environmental conditions under which they live (LIBERG; SANDELL, 1988).

Spraying in domestic cats is recognized by its characteristic behavioural sequence, described by Dehasse (1997) as “approaching a spot with a vertical element – sniffing, sometimes flehmen – kneading activated by a soft surface – turning the back to the vertical element – tail raising – mydriasis – back arching and tail tremor – horizontal *spraying* of urine – turning – sniffing – moving away”. Urine is commonly deposited on prominent conspicuous surfaces and this, together with the fact that within urine there are chemicals which carry information relating to individual

characteristics (e.g. sex, age) and presence in the territory (NATOLI, 1985, 1989), leads to the hypothesis of urine *spraying* serving multiple purposes, from spatial to social marking and reproductive stimulation, for owned cats. Individual differences in *spraying* showed by domestic feral cats may thus reflect “status” and/or “territoriality” (FELDMAN, 1994).

Given its unpleasant consequences for the human beings, urine *spraying* by domestic owned cats is perceived as an unacceptable behaviour to the owners (LANDSBERG, HUNTHAUSEN, ACKERMAN, 1997; HORWITZ, 2002; BOWEN; HEATH, 2005). It is, as a consequence, a common reason for cat relinquishment (SALMAN et al., 2000). *Spraying* is indeed a common behaviour in sexually intact cats (HORWITZ, 2002; CALIXTO; JUSTEN, 2007), however, as many as 10% of castrated males and 5% of neutered females also *spray* on a frequent basis (HART; COOPER, 1984). Clearly, underlying factors transcending the role of sexual hormones, seem to play a role in development and perpetuation of urine *spraying* in domestic owned cats.

In the home, when the purpose is to scent mark, cats commonly deposit urine on significant areas such as near windows and doors or on items with the scent of a particular person or animal (NEILSON, 2004 a). This characteristic supports the argument in favor of a social and spatial function of *spraying* in domestic owned cats. Indeed, most cats exhibiting urine *spraying* are from multi-cat households (OLM; HOUP, 1988; PRYOR et al., 2001) where space is restricted and contact with others is hard to avoid. In a hostile social environment, *spraying* may be used as an attempt to increase distance thus preventing overt confrontation (BOWEN; HEATH, 2005). As found by Skeritt and Jemmett (1980), the larger the number of cats in the house the greater the incidence of urine *spraying*.

Another finding in support of the above regards environmental changes in either physical (e.g. addition of a new furniture) or social (e.g. change in the owner’s schedule) aspects, which commonly lead to urine *spraying* in cats (OLM; HOUP, 1988). As another way of example, domestic owned cats, either housed singly or in multi-cat houses, when presented with separation anxiety syndrome, frequently urine mark on the owners bed (SCWARTZ, 2002). Urine *spraying*, therefore, is more

than an attempt by the cat to control both its space and social relationships and can be a form of exteriorizing and maybe getting relief from internal emotion states. Schwartz (2002) and Bowen and Heath (2005) propose *spraying* is reassuring for the cat who displays it: urine deposits may increase home security thus alleviating anxiety and distress (SCHWARTZ, 2002; BOWEN; HEATH, 2005).

In the clinical context, *spraying* by domestic cats has long been distinguished from inappropriate *toileting* (another “problematic” behaviour characterized by a cat’s failure to use the appropriate latrine for urination and/or defecation) on the basis of its form (LANDSBERG, HUNTHAUSEN, ACKERMAN, 1997; HORWITZ, 2002; BEAVER, 2003; NEILSON, 2004 a,b; BOWEN; HEATH, 2005; HORWITZ; NEILSON, 2007; HERRON, 2010). For instance, *spraying* commonly involves small quantities of urine deposited on vertical surfaces (or on significant horizontal spots) with the cat typically in a standing posture (HORWITZ, 2002; NEILSON, 2004 a,b). “Sprayer” cats generally keep use of the appropriate latrine for both urination and defecation (HORWITZ, 2002; NEILSON, 2004 a,b). Differently, inappropriate *toileting* is mostly characterized by large amounts of urine on preferable horizontal surfaces with cat in a squatting posture (HORWITZ, 2002; NEILSON, 2004 a,b). “Toileter” cats frequently give up using the latrine thus both urine and faeces are commonly found on inappropriate locations (HORWITZ, 2002; NEILSON, 2004 b).

In spite of several clinical behaviour guidelines proposing the aforementioned differentiation, in order to achieve an accurate diagnosis leading to an effective treatment plan, some of the criteria have been challenged. For instance, frequent reports by owners of “sprayer” cats of large amounts of urine on vertical surfaces (CALIXTO; JUSTEN, 2007) challenges the common assumption that these cats eliminate small quantities of urine. In the same direction, a study conducted by Neilson (verbal information)² found that quantities of urine deposited during *spraying* and inappropriate *toileting* did not significantly differ.

Differentiation between *spraying* and inappropriate *toileting* goes beyond their presentation. For a successful treatment, whenever possible, the identification of

² Verbal information provided by J.Neilson at the International Veterinary Behaviour Meeting (IVBM), Edinburgh (Scotland), 2009

underlying causes of the behaviour (e.g. the cat's motivation), which differs in the two forms, is recommended (LANDSBERG, HUNTHAUSEN, ACKERMAN, 1997; HORWITZ, 2002; BEAVER, 2003; NEILSON, 2004 a,b; BOWEN; HEATH, 2005; HORWITZ; NEILSON, 2007; HERRON, 2010). Whilst inappropriate *toileting* is often related to litterbox management, with "toileter" cats possibly presenting preferences and/or aversions to several aspects of the elimination location and soiled areas; anxiety brought about by a threat to local resources, arising from either a change in the physical environment or threat to these resources from another cat, on the other hand, is commonly listed as a major underlying cause of urine *spraying* (HORWITZ, 2002, NEILSON, 2004 a).

Some of the above mentioned features, however, have also been challenged. For example, in a study by Pryor et al. (2001), a treatment regimen for *spraying* cats based on improved cleaning of litterbox and soiled areas besides provision of sufficient number of litterboxes significantly decreased urine marks detected by the owners, specially in the female "sprayers". By contrast the differentiation of *spraying* and inappropriate *toileting* in terms of causative factors (HORWITZ, 2002; NEILSON, 2004 b, HERRON, 2010) mentioned above means such an effect would be expected in cases of inappropriate *toileting* only.

Another questionable aspect concerns the existence of anxiety and distress in *spraying* cats. Despite the attempt by Frank, Erb and Houpt (1999), cortisol:creatinine ratios did not serve to identify distress; even though their average values were 9.9 (healthy normal cats averaged 5.9 in another study – HENRY et al., 1996), a clearly exceptional cat measuring 96.7 certainly contributed to the higher levels of cortisol:creatinine in their *spraying* cats. Excluding the *outlier* cat, average levels decreased to 6.92 (FRANK, ERB; HOUP, 1999).

Common assumptions made in relation to both characterization and causes of urine *spraying* in domestic owned cats therefore need to be reviewed. In this sense, other not so frequently mentioned factors such as cat personality and social behaviour should also be included in a more profound investigation. It is not only suggested that timid (WESTROPP; BUFFINGTON, 2004; BOWEN; HEATH, 2005), or sometimes dominant (CALIXTO; JUSTEN, 2007) cats are at risk of manifesting

spraying but also management of the cat's social status has been included in treatment plans for these cases, even in the absence of empirical data to support the influence of such factors on urine *spraying*.

Finally, more investigation on the presence of concurrent diseases among *spraying* cats is required. In spite of evidences of medical problems in these cats (HORWITZ, 1997; FRANK, ERB; HOUP, 1999) and the overall recommendation of adequate medical screening in order to eliminate medical causes in any case of inappropriate urination (LANDSBERG, HUNTHAUSEN, ACKERMAN, 1997; HORWITZ, 2002; BEAVER, 2003; NEILSON, 2004 a,b; BOWEN; HEATH, 2005; HORWITZ; NEILSON, 2007; HERRON, 2010), some still suggest that neither urination of cats with urinary problem resembles urine marking nor urinalysis provide any relevant diagnostic information in cases of urine *spraying* (TYNES et al., 2003). As proposed by the authors, in cases of urine marking, clinicians should focus on behavioural history, leaving urinalysis to rule out urinary disorders in inappropriate *toileting* cases instead (TYNES et al., 2003). Such advice seems inappropriate as a more complete evaluation of the urinary system (e.g. via ultrasound or cystoscopy) may reveal abnormalities that would not be seen otherwise (CHEW; BUFFINGTON, 2000), thus leading to a mistaken diagnosis. *Spraying*, indeed, might be linked to disease (e.g. painful voiding associated with crystalluria may lead to a standing posture being adopted and small amounts eliminated at a given time). Alternatively, *spraying* in ill cats might be associated with the apprehensive state derived from the ill status itself.

This study aimed at performing an in-depth medical, physiological and behavioural evaluation of cats from multi-cat households presenting with urine *spraying*. In order to identify factors associated to the cat (as opposed to its environment) control cats from the same household were recruited thus constituting a case-control type of study (SCHLESSELMAN, 1982). The idea was a detailed investigation of common assumptions as well as new aspects possibly relating to underlying causes of urine *spraying*. These were: diseases, stress, personality, emotionality and inter-cat social behavior.

3.2 MATERIAL AND METHODS

As described in Material and Methods.

3.3 RESULTS

3.3.1 THE DYADS

The distribution of sex and breed among “case” and “control” cats was not significantly different (Exact Fisher test - sex: $p=0.183$; breed: $p>0.999$); “case” and “controls” also did not significantly differ as a function of their age (Student paired t-test, $p=0.889$). These are in accordance with our procedure of matching “case” and “controls” on the basis of these variables, whenever possible.

3.3.2 MEDICAL ASPECTS

Preliminary Exams

Preliminary exams which determined selection of the dyads kept in the study revealed that out of 18 apparent healthy cats exhibiting urine *spraying*, 7 (i.e. 38.9%) demonstrated a medical problem; as for their controls, this was found in only one cat (out of 18 = 5.6%). Significantly more “case” than “control” cats (Fisher exact test, $p=0.041$) therefore had one of the following medical conditions: renal insufficiency (2), idiopathic cystitis (2), diabetes insipidus (1), diabetes mellitus (1), ectopic testes (1). The only excluded control cat had a lithiasis within the bladder.

The remaining 11 dyads included in the study were considered to be healthy on the basis of the initial health screen; this initial screen did not exclude some medical complications which did not obviously impair either urinary or other organic system functioning. These conditions were identified: four “case” and one “control” cat presented renal lithiasis (these measured about 0.3 cm and in all cases were located at the renal pelvis); a renal cystis was found in a fifth “case” cat. One “case” and one “control” showed renal diverticulum calcification.

This meant that in total 12 out of the initial 18 recruited “sprayers” (i.e. 66.7%) had medical abnormalities. In relation to the “controls”, 3 out of 18 (i.e. 16.7%) had alterations seen at the medical check-up. Among them, however, those with normal functioning were selected for the study together with those that had completely clear results.

One of the selected “case” cats showed urine excretion of high amounts of glucose. Blood levels of glucose as well as fructosamine were within normal reference range, though. Leucocytes count was high (i.e. 19,900 cells/mm³). Further urinalysis did not present glycosuria thus excluding any suspicions of diabetes mellitus.

Counts of blood leucocytes of “cases” and “controls” did not significantly differ (i.e. median: “case” cats = 13,700.00 cells/mm³ [IQR = 3,800.00; n=11], “control” cats = 10,700.00 cells/mm³ [IQR = 13,200.00; n=11]; Wilcoxon signed-rank test: p = 0.6377). However, serum creatinine levels were significantly higher for *sprayers* in comparison with their “controls” (i.e. mean: “case” cats = 1.53 mg/dL [SD= 0.31; n=11], “control” cats = 1.19 mg/dL [SD= 0.23; n=11]; Student paired t-test: p=0.005). With regard to urea, the difference between “cases” and “controls” was nearly significant (i.e. mean: “case” cats = 62.68 mg/dL [SD=6.13, n=11], “control” cats = 56.35 mg/dL [SD=6.41, n=11]; Student paired t-test: p=0.0597).

Cystoscopy

Cystoscopy performed in one of the two selected *spraying* female cats revealed several spreaded submucosal petechiae (“glomerulations”) (figure 12), edema and mild bladder haemorrhage, thus leading to a diagnosis of interstitial cystitis.

Follow-up: on the days following cystoscopy the cat showed hematuria, polakiuria and an apparently painful urination. A *toileting* type of inappropriate urination arose with the cat depositing urine containing blood in the bathroom sink (such a behaviour had never been presented before). This lasted a couple of weeks and gave the impression to the owner that problem significantly worsened after cystoscopic examination. The cat was successfully treated with amitriptyline (1mg/kg, BID) from the first day after cystoscopy for 60 days; the cat returned to using the litterbox for urination with the owner reporting only rare occasions of urine *spraying*. Such improvement was sustained after 6 months when the last contact with the owner occurred.

Figure 12. Cystoscopic examination of a female “sprayer” cat. White arrows indicate petechiae (A – normal size; B – 1.5X zoom).

3.3.3 PHYSIOLOGICAL ASPECTS

Faecal glucocorticoid metabolites

The owner of one dyad (*Mitchu-Et*) was unable to collect faeces of the cats as she never witnessed defecation of the participant cats. Results of the remaining 10 “case-control” dyads are therefore presented.

Whilst “sprayer” cats had a median level of faecal glucocorticoid metabolites equal to 496.70 ng/g dry faeces (IQR = 497.75; n=10), median level of their “controls” was 545.34 ng/g dry faeces (IQR = 325.42; n=10). One “case” cat (*Mussum*), and one “control” cat (*Macaca*) had levels clearly different from the rest of the group (i.e. *Mussum*= 1,846 ng/g dry faeces; *Macaca*=1,894 ng/g dry faeces) and thus could be considered as potential *outliers* (figure 13). However, given the large variation in glucocorticoids levels, and the fact that other cats approximated to the *outliers* in their values, we decided to keep them for the following inferential analysis. Shapiro-Wilk normality test revealed distribution of the variable significantly different from normal for “case” but not for “control” cats (p=0.023 and p=0.097, respectively). Faecal glucocorticoid metabolites did not show significant differences when “case” and “control” cats were compared (Wilcoxon signed-rank test, p=1.000).

Figure 13. Faecal glucocorticoids metabolites for the 10 “case-control” SPRAYING dyads.

Plasma bioamines

Blood sample of one “case” cat (*Guto*) was only sufficient to perform a biochemistry profile and blood count; bioamines analysis could not be performed. Data from its “control” cat (*Thiago*) were also excluded, results of the remaining 10 “case-control” dyads are presented.

Tubes test – Levels of serotonin in 7 shelter cats used for assessment of the validity of the method did not correlate when values derived from the same samples stored in EDTA tubes were compared with the ones in dry tubes. Likewise, levels of epinephrine and norepinephrine (but not dopamine) did not correlate when values from EDTA tubes were compared with the ones in heparin tubes. Intra-class correlation coefficients (ICC) were as follows: serotonin (ICC=0.351, $p=0.180$); dopamine (ICC=0.781, $p=0.012$); epinephrine (ICC=0.147, $p=0.351$); norepinephrine (ICC=0.039, $p=0.454$). The more ICC is close to one, the more similar are the values from the two methods (i.e. two different tubes for samples storage). There is thus only significant similarity for dopamine values taken from samples stored in EDTA and heparinised tubes.

Duplicate samples test – Levels of serotonin and epinephrine (but not dopamine and norepinephrine) in the 5 owned cats did not correlate when values taken from duplicate eppendorfs derived from the same samples stored in EDTA tube were compared. Intra-class correlation coefficients (ICC) were as follows: serotonin (ICC=0.00, $p=0.915$); dopamine (ICC=0.714, $p=0.038$); epinephrine (ICC=0.00, $p=0.664$); norepinephrine (ICC=0.962, $p< 0.001$). The more ICC is close to one, the more similar are the values from the two eppendorfs (same EDTA stored sample). There is thus only significant similarity for dopamine and norepinephrine values taken in duplicate from EDTA tubes.

Plasma levels of serotonin, epinephrine, dopamine and norepinephrine are presented in figure 14.

Shapiro-Wilk normality test revealed dopamine distribution significantly different from normal for “case” but not for “control” cats ($p=0.005$ and $p=0.154$,

respectively). Plasma levels of dopamine were not significantly different between “cases” and “controls” (Wilcoxon signed-rank test, $p=0.084$).

Figure 14. Plasma bioamines for the 10 “case-control” SPRAYING dyads.

	Serotonin (ng/ml)	Dopamine (pg/ml)	Epinephrine (pg/ml)	Norepinephrine (pg/ml)
“CASES”	N=10	N=10	N=10	N=10
<i>Mean</i>	173.01	157.15	200.11	495.44
<i>Median</i>	139.91	108.30	188.40	378.45
<i>Minimum-Maximum</i>	1.43-696.91	66.13-407.84	100.13-310.89	117.41-987.12
<i>Standard Deviation</i>	199.18	119.84	75.32	342.49
<i>Inter-quartile Range</i>	142.52	106.73	147.86	662.83
“CONTROLS”	N=10	N=10	N=10	N=10
<i>Mean</i>	355.91	214.25	181.15	412.51
<i>Median</i>	204.34	173.71	158.95	315.92
<i>Minimum-Maximum</i>	29.33-1,281.66	35.85-410.31	45.52-417.19	135.81-1,431.94
<i>Standard Deviation</i>	403.10	139.16	109.63	378,38
<i>Inter-quartile Range</i>	502.59	258.28	129.31	214.09

3.3.4 BEHAVIOURAL ASPECTS

Sociality

According to the owners’ opinion about their cats levels of dominance and submissiveness in relation to the other members of the group (both in a scale from 0 to 10, being 0=nothing and 10=totally), “case” and “control” cats did not significantly

differ as to their *dominance scores* (Wilcoxon signed-rank test, $p=0.3906$; median=2.00 [IQR=3.50] and 0.50 [IQR=2.50]), respectively for “cases” and “controls”, $n=9$ dyads) nor their *submissiveness scores* (Student paired t-test, $p=0.233$; mean=2.17 [SD=2.29] and 4.28 [SD=3.85], respectively for “cases” and “controls”, $n=9$ dyads). A *dominance index* composed of the *dominance scores* minus *submissiveness scores*, from the same questionnaire based scales, also did not show any significant difference when “case” and “control” cats were compared (Student paired t-test, $p=0.262$; mean=1.39 [SD=4.94] and -2.39 [SD=5.30], respectively for “cases” and “controls”, $n=9$ dyads).

Regarding comparative analysis of the Dominance Trees derived from the agonistic interactions between the cats and reported by the owners, both *cat's rank* and *number of cats which it dominates* did not significantly differ between “case” and “control” cats (Wilcoxon signed-rank test, $p=0.671$ and $p=0.828$, respectively, $n=10$ dyads). Whereas *spraying* cats had median rank of 2.50 (IQR=2.00) and dominated a median of 0.00 individuals (SD=2.00), their “controls” had *tree rank* median of 2.50 (SD=1.00) and dominated a median of 1.00 cats (SD=2.00). As to overall *frequency of agonistic interactions* within the group, “sprayers” and “controls” also did not differ significantly (“sprayers”: mean=5.64, SD=5.66; “controls”: mean=4.91, SD=4.28; Student paired t-test, $p=0.693$, $n=11$ dyads).

As it can be seen in an example of Dominance Tree (figure 15) constructed for the *Guto-Thiago* household, the lower the *cat's rank* value the more dominant within the group the cat is. Cat's rank is on the left with number of dominated individuals in parentheses. Dominance trees for the other households can be seen in Appendices G-O.

Regarding comparative analysis of the Affiliative Trees derived from the affiliative interactions between the cats and reported by the owners, the overall *frequency of affiliative interactions* did not significantly differ between “case” and “control” cats (Wilcoxon signed-rank test $p=0.343$, $n=11$ dyads). Whereas *spraying* cats had median frequency of 5.00 (IQR =39.00) their “controls” had median frequency of 15.00 (IQR=36.00).

As it can be seen in an example of Affiliative Tree (figure 16) constructed for the *Guto-Thiago* household, the shorter the lines between the cats the stronger the

affiliation between them. Such a distance calculated from the reversion of their frequencies are provided in the lines. Affiliative Trees for the other households can be seen in the Appendices G-O.

Figure 15. Dominance Tree for the household containing the “case-control” dyad *Guto-Thiago* (dashed line indicates the “control” cat). Male cats are represented in blue and female cats in red.

Figure 16. Affiliative Tree for the household containing the “case-control” dyad *Guto-Thiago* (dashed line indicates the “control” cat). Male cats are represented in blue and female cats in red.

Personality

Out of the 9 owners (i.e. there were 11 dyads, but nine households), only one did not complete the personality questionnaire for the participant cats. As for the reliability tests, in 5 households there was other family member to answer the questionnaires.

Reliability of owners' opinions, measured through correlations between their scores and other family members' scores, revealed that as for “timid” there was significant similarity (intra-class correlation = 0.622, $p=0.016$). As for “bossy” and “easy-going” correlations were weak (intra-class correlation = 0.392 and 0.068,

$p=0.106$ and $p=0.414$, respectively). However, considering the inter-rater correlation suggested by Meagher (i.e. at least 70%), scores for the three personalities were considered unreliable.

Whilst *spraying* cats had median scores of 4.74 for “bossy” (IQR=8.50), of 5.00 for “timid” (IQR=5.50) and of 5.50 for “easy-going” (IQR=5.00), their “controls” had median scores of 2.25 for “bossy” (IQR=6.00), of 3.00 for “timid” (IQR=8.00) and of 7.75 for “easy-going” (IQR=3.00).

Regarding evaluation of reliability of owners’ opinion on the seven behavioural domains adapted from the work of Feaver, Mendl and Bateson (1986) (“active”, “curious”, “sociable with people”, “hostile to people”, “tense”, “fearful of people”, “equable with cats”), this was taken through comparisons between their opinion about the behaviour of cat’s in general taken in two moments: first whilst scoring about “case” cats and second while answering about “control” cats.

A significant similarity was found for “curious” (intra-class correlation=0.919, $p<0.001$), “sociable with people” (intra-class correlation=0.950, $p<0.001$), “fearful of people” (intra-class correlation=0.848, $p<0.001$), “hostile to people” (intra-class correlation=0.848, $p<0.001$) and “tense” (intra-class correlation=0.779, $p=0.002$) but not for “active” (intra-class correlation=0.341, $p=0.142$) and “equable with cats” (intra-class correlation=0.470, $p=0.064$). Applying the suggestion by Meagher (2009) also for intra-rater correlation (i.e. at least 70%), the same domains could be considered reliable.

“Case” and “control” cats did not significantly differ in the five domains for which we gathered reliable scores (Student paired t-test, $p=0.310$ [curious], $p=0.741$ [sociable with people], $p=0.596$ [fearful of people], $p=0.662$ [hostile to people], $p=0.380$ [tense]).

Mean scores by the owners for each of the 7 behavioural domains accompanied by the respective standard deviations, for “case” and “control” cats, can be seen in figure 17.

Figure 17. Owners' score in the 7 behavioural domains for the 10 "case-control" SPRAYING dyads.

	Active	Curious	Sociable with people	Hostile to people	Tense	Fearful to people	Equable with cats
CASES	N=10	N=10	N=10	N=10	N=10	N=10	N=10
<i>Mean</i>	-0.10	1.10	1.40	-1.40	-0.20	-0.65	-0.25
<i>Median</i>	0.50	2.00	1.00	-1.00	-1.00	0.00	0.00
<i>Minimum- Maximum</i>	-8.00- 5.00	-6.00-4.00	-5.00-6.00	-6.00-2.00	-6.00-5.00	-6.00-5.50	-5.00-3.00
<i>Standard Deviation</i>	4.07	3.11	3.69	2.67	3.16	3.68	2.32
<i>Inter-quartile Range</i>	7.00	3.00	3.00	3.00	3.00	4.00	2.00
CONTROLS	N=10	N=10	N=10	N=10	N=10	N=10	N=10
<i>Mean</i>	-1.10	0.10	0.90	-1.90	-1.50	-1.35	0.20
<i>Median</i>	-1.00	0.00	1.00	-1.50	-1.00	-1.50	-0.50
<i>Minimum- Maximum</i>	-5.00- 4.00	-4.00-7.00	-4.00-7.00	-6.00-2.00	-8.00-4.00	-8.00-4.50	-4.00-5.00
<i>Standard Deviation</i>	2.64	2.96	3.96	2.81	3.31	3.71	3.19
<i>Inter-quartile Range</i>	4.00	1.00	7.00	4.00	3.00	4.00	5.00

Videos – Analysis of videos from 18 cats (i.e. 9 dyads) were performed. Videos from the three remaining dyads had to be excluded due to owners interference (e.g. taking the cat out of the hidden place, picking the cat onto the lap, etc.), despite clear instructions not to do so.

First 3 minutes – most cats spent a great proportion of the time in the hidden place (“cases” –median=131.60s, IQR=89.00; “controls” –median=108.40s, IQR=133.20). There was not a significant difference between “cases” and “controls”

as to either time spent hidden (Wilcoxon signed-rank test, $p=0.843$) or in close proximity with the owner (i.e. Zone 1 + Zone 2 + under the owners' chair = median of 2.20s [IQR=31.40] versus 8.40s [IQR=51.00]; Wilcoxon signed-rank test, $p=0.4609$) and far from the owner (i.e. Zones 3 + Zone 4 + out of view = mean of 43.20s [IQR=4.00] versus 41.40s [IQR=16.00]; Wilcoxon signed-rank test, $p=0.480$).

None of the cats stayed *on top of its carrier* nor *onto the owner's lap*. Whilst only one "case" cat jumped onto the *table* and none into the *sink*, only one "control" cat jumped into the *sink* and none onto the *table*.

About the *far from owner/(far + close) from owner* proportion, "case" cats did not significantly differ to the "controls" ("cases" - median was 4.68%, IQR=38.51; "controls" - median was 17.14%, IQR=49.92, Wilcoxon signed rank test, $p=0.425$) thus indicating that, "cases" and "controls" spent similar time distant (proportionally to close) from the owner.

As to *looking owner* and *investigating owner*, there was also not significant differences between "cases" and "controls" (Wilcoxon signed-rank test, $p=0.578$) considering the two behaviours together. Whilst "cases" spent about 1.40s (IQR=16.60), "controls" spent about 0.00 (IQR=1.00) on this composite behaviour. Significant differences were also not found when *looking owner* plus *investigating owner* behaviour was considered in terms of its frequency ("cases": median = 1.00 times, IQR=3.00; "controls": median = 0.00 times, IQR=1.00; Wilcoxon signed-rank test, $p=0.703$).

Investigate carrier was performed by 4 "cases" and 2 "controls".

As to *meowing*, which was only measured in terms of its frequency, "cases" and "controls" also did not significantly differ: whilst *spraying* cats meowed a median of 7.00 times (IQR=26.00, $n=6$), their "controls" meowed a median of 0.00 times (IQR=9.00, $n=4$) (Wilcoxon signed-rank test, $p=0.437$).

Concerning time spent *stopped* versus *moving*, whilst *spraying* cats spent on average 5.80s (IQR=32.00) moving and 1.00s (IQR=3.40) stopped (always in *stand up* posture), their "controls" spent on average 2.00s (IQR=18.80) moving and 0.40s (IQR=90.40) stopped (in *sit*, *lie down*, *cower* or *stand up* postures) (Wilcoxon signed-rank test, $p=0.359$ [moving] and $p=0.375$ [stopped]).

About the *move/(move + stop)* proportion, “cases” and “controls” did not significantly differ: “cases” - median was 87.88%, IQR=28.17s; “controls” - median was 64.32%, IQR=77.45, Wilcoxon signed-rank test, $p=0.312$) thus indicating that, “cases” and “controls” spent similar time moving (proportionally to stopped) around the room. *Moving overt frightened* which was measured and analysed separately from general movement was only performed by one “control” cat.

The remaining 2 minutes (with food) - 3 “case” and 2 “control” cats ate the food provided. Whilst the three “cases” took on average 0.8s (median) to eat food, the two “controls” took about 1.8s (median). Food was eaten only once for all the cats.

3.4 DISCUSSION

Medical Aspects

“Sprayers” in this research were mostly males, with only two females exhibiting the problem. Indeed, as with other felid species (SCHALER, 1972), female feral cats are rarely seen urine *spraying* (FELDMAN, 1994). Our results also conform to previously published research of urine *spraying* in which males frequently outnumbered females to as much as 10 to 1 (e.g. OLM; HOUP, 1988; PRYOR et al., 2001; TYNES et al., 2003; HART et al., 2005; LANDSBERG; WILSON, 2005). Although feral females have been seen urine *spraying* in both reproductive and non-reproductive contexts (PANAMAN, 1981; NATOLI, 1985) and indeed, even at lower rate, gonadectomised female owned cats urine mark, one may hypothesized that some of the functions *spraying* holds for male cats might be of marginal importance to females. As found by FELDMAN (1994), other scent mark forms such as *scratching* are commonly displayed by females; these could, for instance, substitute *spraying*, at least in parts, on its social, spatial and stress relief functions.

A significantly higher occurrence of overt disease was found for selected “sprayers” in comparison to their “controls” (i.e. 38,9% versus 5.6%) thus indicating that, at least for some apparently healthy cats, *spraying* might be a sign of illness. As for the “controls”, our results corroborate a previous report of 100 apparently clinically healthy cats in which 6% had medical problems (e.g. hyperthyroidism, urinary infection) (GARCIA; BRUYETTE apud LANDSBERG; WILSON, 2005). The presence of medical conditions among our selected “sprayers” was higher than a previously published research (LANDSBERG; WILSON, 2005), but similar to another (FRANK, ERB; HOUP, 1999). Such differences are probably due to which medical conditions are considered as possibly interfering with the urination act. In the study reported here, any alteration of the upper urinary system compromising urinary function, any abnormality of the lower urinary tract or altered functioning of the organism in general were considered as possibly linked to *spraying* behaviour thus leading to the cat (and therefore the dyad) not being recruited. By contrast, other studies have only considered lower urinary tract disorders or the sole result of urinalysis as exclusionary criteria (LANDSBERG; WILSON, 2005; TYNES et al., 2003).

The criteria from these other studies, however, do not seem to be as appropriate to rule out medical causes in urine *spraying* cases. Firstly, some conditions altering the urinary system could be identified, for instance, only at imaging exams such as ultra-sound or even endoscopy of the urinary system (BUFFINGTON, CHEW; DIBARTOLLA, 1996; CHEW; BUFFINGTON, 2000). Secondly, problems in the upper urinary tract such as chronic kidney disease (CKD) (ROUDEBUSHI et al., 2009; WAKI et al., 2010, 2006) can interfere with urinary functioning thus leading to inappropriate urination and yet be not accompanied by alterations at urinalysis.

Despite the common assumption that irritable voiding or increased urination (as either pollakiuria or polyuria) are likely to cause inappropriate *toileting* behaviour (HOSTUTLER, CHEW; DIBARTOLLA, 2005), there have been reports of cats in which *spraying* behaviour does seem to resemble urinary disease, for instance in situations in which they *spray* bloody urine (WESTROPP; BUFFINGTON, 2004).

Selection criteria in this study were thus strictly controlled with regard to medical components. Nonetheless, supposedly “healthy” selected cats presented medical conditions which could constitute either co-morbid or linked to the urine *spraying* states. For instance, whilst four recruited “sprayer” cats had renal lithiasis, one had a renal cyst; conversely, only one “control” cat had renal lithiasis. Since signs of renal mal functioning (seen by a combined interpretation of their urinalysis and serum creatinine levels) were absent, these cats were kept in the study. At least in their cases, and at that moment in time, renal formations were considered unlikely to be interfering with urination behaviour.

Another important finding was the significantly higher creatinine and urea levels among “case” cats in comparison with their “controls”. Serum creatinine values in the “cases” ranged from 0,89 to 2,00 mg/dL (whereas values for urea varied from 55,9 to 72,10 mg/dL). One could argue that on the basis of creatinine, at least three “case” cats with values beyond 1,6 mg/dL should be considered as having CKD-stage 2 (classification system by the International Renal Interest Society – IRIS, mentioned by ROUDEBUSHI et al., 2009; WAKI et al., 2010). Indeed, these three cats are among the ones previously reported with renal lithiasis/cyst. Since renal lithiasis is progressively seen in association with CKD in cats (PIMENTA et al., 2011) and suggested as either a complication or a CKD cause, it may be that these cats were indeed CKD patients.

While the above assumption may hold true, the finding that their urine specific gravities were all beyond 1.030 and the absence of other signs of CKD (apart from the non-specific higher levels of urea), leads to the following two hypotheses for the above mentioned three cats: (A) they were at early CKD-stage 2 yet with no implications for the urinary function, (B) high creatinine levels only reflected a momentum or normality for them (with renal formations been just co-morbid states). As recommended by the International Renal Interest Society (IRIS), creatinine should be assessed on at least two occasions in a stable patient before considering CKD. Indeed, in a further biochemistry repetition in one of the three cats, creatinine decreased to 1,2 mg/dL (it was 2,0 mg/dL at the time of the study).

Whereas strong evidence of higher creatinine/urea levels interfering with urination via renal failure were lacking, a direct link between renal formations and urine *spraying* via pain could still be argued. When ruling out medical causes in cases of inappropriate urination, attention is given to the lower urinary tract (HORWITZ, 2002; NEILSON, 2004 a,b; HERRON, 2010); upper urinary tract imaging, among others, is commonly considered as a further possible test, but restricted to cases on the basis of alterations in essential first required exams such as urinalysis, biochemical profile and complete blood count (HORWITZ, 2002, NEILSON, 2004 a,b; BOWEN; HEATH, 2005; HERRON, 2010).

Our findings concerning renal formations and increased urea/creatinine levels may be simply incidental due to our small sample size. On the other hand, it may raise some questions such as whether *spraying* maybe a precocious sign of renal malfunctioning; besides, could renal formations interfere directly with the urination act via pain or discomfort? For instance, if renal calculi cause pain, a change from a squatting to a standing posture may be a relief strategy. All those five *spraying* cats had formations located at the renal pelvis, which in the case of human lithiasis leads to pain in some (but not all) patients (HILL et al., 1985). Further investigation of behavioural indications of pain (BENNETT; MORTON, 2009) in cats presenting with renal formations is therefore required.

Renal calculi were also detected in a previous study including radiography of the urinary system as part of the medical check-ups for cats presenting with urine *spraying* (FRANK, ERB & HOUP, 1999). Based on the present results, and despite the suggestion by TYNES et al. (2003) to focus on behavioural history as opposed to medical investigation in cases of urine *spraying*, emphasis should be made on the need for a complete medical check-up, including upper urinary tract imaging and biochemistry profile, for any case of urine *spraying*.

Our most relevant medical finding was the diagnosis of interstitial cystitis in one *sprayer* female cat. Pathognomonic submucosal petechiae (i.e. “glomerulations”) (CHEW; BUFFINGTON, 2000; HOSTUTLER, CHEW; DIBARTOLLA, 2005) together with bladder edema were considered for the definite diagnosis; haemorrhage was thus likely to be a consequence of procedural bladder distension (MESSER, CHEW;

MCLOUGHLIN, 2005). As suggested by Chew and Buffington (2000), without cystoscopy, several cases of inappropriate urination are at risk of being erroneously diagnosed as a purely behavioural problem. Indeed, in the case of this female cat, results within normality in all the other previous exams together with the lack of clinical signs, had led us to diagnosis of an exclusive behavioural condition.

It ought to be considered, however, that cystoscopy was performed some time after the other exams and so we cannot know whether alterations would be seen on those exams if they were performed on the day of cystoscopy. As best described by Neilson (2004 a) feline lower urinary tract diseases can progress as a “waxing and waning” processes, thus making clinical signs as well as laboratory and imaging findings transient. In this sense, and regarding interstitial cystitis, cystoscopy is a very appropriate investigation tool as compatible bladder alterations are seen even in patients without signs of the disease (RECHE-JUNIOR; HAGIWARA, 2001). The suggestion by some authors that submucosal petechiae are nonspecific (RECHE-JUNIOR; HAGIWARA, 2001, 2005) and could be even a result of the cystoscopic procedure itself (JOHANSSON; FALL, 1990) cannot be excluded. In our case, the fact that other alterations such as bladder edema were present and other possible causes of petechiae (e.g. infection, neoplasia) had been excluded, supports our diagnosis, though. Additional concerns relating to procedural risks and post-cystoscopy complications, however, deserve further attention.

Physiological aspects

Considering faecal glucocorticoids metabolites as physiological parameter of stress, support for the traditional claim about *spraying* cats being anxious or distressed (LANDSBERG, HUNTHAUSEN; ACKERMAN, 1997; HORWITZ, 2002, NEILSON, 2004 a,b; BOWEN; HEATH, 2005, HORWITZ; NEILSON, 2007) were not found in this study. Once more (FRANK, ERB; HOUP, 1999), evidence for the hypothesis of *spraying* being a feline stress reaction remains to be demonstrated.

It may be that our “*sprayer*” cats are as relaxed as their controls; conversely, they could be as stressed as the “non-sprayers”. Evidences of the latter exist and come from a comparison to previously published research with laboratory cats whose faecal glucocorticoids levels (measured by the same EIA-oxoacetylcholonalone) were far below values found in this study (PALME, SCHATZ; MOSTL, 2001; SCHATZ; PALME, 2001). For instance, whilst their median baseline levels was 196 ng/g (SCHATZ; PALME, 2001), ours was 517.53 ng/g (values considering both “case” and “control” cats). In fact, some of our cats (“mussum”, “macaca”) reached levels seen in cats after ACTH injection (SCHATZ; PALME, 2001). It ought to be considered that our extraction method used a more concentrated alcohol (90% methanol as opposed to their 80% methanol – SCHATZ; PALME, 2001; PALME, SCHATZ; MOSTL, 2001) which was thus expected to result in more glucocorticoids being extracted. The increment was predicted to be about 5% only, though.³ Clearly, higher levels found here were not due to a more powerful extraction method.

In a recent unpublished study by our research team, faecal glucocorticoids metabolites from domestic owned cats (n=57) coming from multi-cat houses of 3-4 cats (n=17) were measured. Median levels found through a 90% methanol extraction followed by EIA-oxoacetylcholonalone dosages was 227.11 ng\g. It is important to note that none of the owners mentioned *spraying* as a behaviour manifested by any of their cats and indeed overt behavioural problems such as severe aggression were considered an exclusion criterion. As it stands, *spraying* behaviour seem to arise in a generally high arousal (probably stressful) environment. As glucocorticoids concentrations of both our “*sprayers*” and “controls” were high, the leading hypothesis is that *spraying* might function as a coping strategy (KOOLHAAS et al., 1999; KOOLHAAS, 2008) which seems to be unsuccessful as values for “*sprayers*” were as high as for the “controls”. However, as we do not know about their previous glucocorticoids levels, we cannot exclude the possibility of even higher levels which decreased with the manifestation of *spraying* behaviour.

³ Verbal information provided by Prof.Rupert Palme in November/2011 at Viena University of Veterinary Medicine.

An interesting finding in the same direction exists concerning *hiding* behaviour by domestic cats (CARLSTEAD, BROWN; STRAWN, 1993). In this case, however, a clear decrease in excreted urinary cortisol was seen in caged laboratory cats that hid themselves behind the litter tray when under a stressful condition (CARLSTEAD, BROWN; STRAWN, 1993).

One may argue that the exclusive measurement of faecal glucocorticoids is not an appropriate tool to investigate stress. Indeed, given the lack of a reference range, our conclusions would be rather limited if we could not compare our results with other studies thus not concluding that overall values for both “cases” and “controls” were high. Another limitation regards the hypothalamic-pituitary-adrenal HPA axis being attenuated under prolonged stress thus not being the ideal tool for measuring chronic stress (KEAY et al., 2006; MORMED et al., 2007). Furthermore, positive stress reaction denoting excitement such as during courtship, sexual behaviour or hunting (MOSTL; PALME, 2002; TOUMA; PALME, 2005) could also be reflected in increased faecal glucocorticoids excretion.

The possibility of such positive stress contributing to the high faecal glucocorticoid values in this study seem to be unlikely as other evidence of distress (e.g. glucosuria in one cat – Mussum) were seen. Importantly, a probable chronic stress state by these cats as a result of long time living in a multi-cat house seemed to be detected by our EIA-oxo method or these cats were being constantly exposed to acute stressors (e.g. inter-cat conflicts) which were probably frequent enough to be picked by faecal dosages.

HPLC method for measuring feline plasma bioamines from samples stored in EDTA tubes (revealed by both *tubes test* and *duplicate samples* tests) demonstrated unreliability for most of the neurotransmitters (the exception was dopamine). This strongly restricts our understanding about what is going on regarding emotionality of “sprayer” cats. If a reliable measurement had been achieved, neurochemical aspects could maybe suggest why “cases” (but not “controls”), although as aroused as the “controls”, urine sprayed. In terms of dopamine levels at least, “cases” and “controls” seemed to be at the same emotional state.

Before any further attempt to measure domestic cats neurotransmitters levels, further studies involving reliability tests of blood stored in heparinised tubes for measuring catecholamines and dry tubes for storing blood samples for serotonin dosages are required. Studies on stability of catecholamines, for instance, recommends that heparinised tubes and immediate centrifugation are preferred (BOOMSMA et al., 1993; DÁLESANDRO et al., 1990). Regarding serotonin, while some still recommend EDTA storage and natural decantation at the refrigerator when collecting plasma (TEKES, 2008), the fact that such proceeding negatively interferes with dosages of catecholamines (BOOMSMA et al., 1993) leads to the question on whether the same may happen with serotonin. Further studies regarding stability of serotonin is therefore required.

Behavioural aspects

According to the owners, “cases” are not significant different to “controls” as to *dominance* and *submissiveness* scores. Even though evidence of reliability and validity properties of their opinions on these two concepts are lacking, results from their scores were in accordance with conclusions from the agonistic matrices thus adding some validity to their scores. “Case” cats were no more involved than “control” cats in agonistic interactions (e.g. biting, hissing, growling, and attacking). Besides, no consistent social position at the Dominance Trees was seen for the “sprayers”. For the construction of these, some aspects of linear dominance hierarchy in the sense that if cat A dominates B, and B dominates C, then A also dominates C, is assumed (IZAR; SATO, 1997; FURNARI, 2011). Linear dominance hierarchies have been previously identified in studies on the social organization in groups of domestic feral cats (NATOLI; DEVITO, 2001; NATOLI, BAGGION; PONTIER, 2001) and so the use of it in this study, as an attempt to detect social patterns in “sprayer” cats, should not be seen as data over interpretation.

In the present study, “sprayers” were sometimes high in the dominance hierarchy, sometimes at the bottom of it, or in between the two extremes. Number of dominated individuals also did not significantly differ between “cases” and “controls”. In spite of previous studies having found correlations between urine *spraying* (among other scent mark behaviours) and dominance status (DE BOER, 1977; LIBERG, 1980; NATOLI, BAGGIO; PONTIER; 2001) in feral cats, this may not be the case for the owned neutered cats living under human mentoring. As suggested by Natoli and DeVito (1991) *spraying* may also act as displacement activity. Indeed, castration clearly decrease urine spraying but its influence on dominance relationships remains to be demonstrated (DE BOER, 1977), thus suggesting that the link between *spraying* and dominance is rather unclear. In any case, *spraying* can still play a role in spatial defence and yet not be related to social dominance.

As for the affiliative interactions, and similar to agonism, “sprayers” were sometimes very close to other cats in some houses, yet distant from the group in others. Frequency of affiliative interaction between “cases” (versus “controls”) and other cats in the group also did not differ significantly. As social hierarchies can also be constructed on the basis of social bonding and some have suggested that the dominant cat is the one receiving higher amounts of amicable behaviour (MCDONALD, 1987; NATOLI, BAGGIO; PONTIER, 2001; CAFAZZO; NATOLI, 2009), our results for affiliation corroborates the ones for agonism thus emphasizing that urine *spraying* does not seem to be linked to social dominance. Likewise, it does not seem to be a plea from a socially isolated individual as it could appear when *spraying* manifested by cats that do not seem to be well fitted into the group; in only one of the households “sprayer” was a socially isolated individual.

Although not tested for reliability, owners observations on social relationships are not so dependent upon interpretation which differs from their opinion on personality. The present results indicate spraying is not linked to cat-cat social behaviour, but do not exclude the possibility of a link between *spraying* and social behaviour in the human-cat level. The multi-cat houses are a particular environment and thus the way in which individuals socially aggregate may be particularly distinct (BRADSHAW; LOVETT,?; PONTIER; NATOLI, 1996); the function of communicative

signals such as scent marking may differ from the ones seen in their feral counterparts.

As to personality, inter-rater reliability test based on a comparison of the owners answers with those from another family member was revealed to be weak. Family members do not strongly agree on how their cats fit with “bossy”, “timid” or “easy-going”. As it is not possible to know whether one or the other, if any, conform with reality, none of the opinions can be reliably taken into consideration. Although no strict rules exist, Meagher (2009) suggests that correlations of 0.7 or greater are desirable.

Similar results were found in regard to the scores for the seven behavioural domains adapted from the work of Feaver, Mendel and Bateson (1986). Significant differences were not found when scores for “cases and “controls” were compared; intra-observer reliability test was strong (i.e. > 0.7 – according to MEAGHER, 2009) for “curious”, “sociable with people”, “fearful of people”, “hostile to people” and “tense”, but not for “active” and “equable with cats”. Despite the definitions provided in the questionnaire, owners may not have understood the question. Whatever the cause, their ratings for their own cats on “active” and “equable with cats” cannot be considered further. As it stands, “sprayers” did not differ to their controls in their reaction to people nor in their levels of curiosity and tenseness. A possible correlation between *spraying* and feline social behavior on the cat-human relationship level should be treated with reservation. As to curiosity and tenseness which might relate to reward sensitivity and fearfulness there appears to be no difference, again suggesting that the difference between sprayers and controls does not lie in their definable personality, contrary to the opinion of some (WESTROPP; BUFFINGTON, 2004; BOWEN; HEATH, 2005).

Some may argue that other more objective temperament test such as the Feline Temperament Profile (FTP) used in previously published research on domestic cat individuality (SIEGFORD, 2004; IKI et al., 2011) could have been used instead. However, considering the extra time which would be spent in the already long visit to the veterinary hospital, together with the fact that cats change behavioural strategy across specific contexts (KIDIE; CASEY, 2009), an integrative evaluation of more

overall patterns based on everyday behaviours rated by people familiar to them seemed to us much more valuable (FEAVER, MENDL & BATESON, 1986; MEAGHER, 2009).

The lack of significant differences between “cases” and “controls” on the behaviours seen in the videos seem to support our conclusion that “sprayers” do not differ on temperament or behavioural style as far as we can tell. Further studies with larger samples size may confirm this. But they do differ in their medical profile which reinforces the importance of medical evaluation in cases of urine *spraying*.

Chapter 2.

"Medical, physiological and behavioural aspects of cats exhibiting **inappropriate toileting behaviour**: a case-control study with multi-cat households"

Abstract

Inappropriate *toileting* behaviour is a common problem seen by behaviour experts and one of the top causes of cat abandonment. Despite clear instructions for behavioural diagnosis and therapeutic regimes proposed by several traditional veterinary behavioural guidelines, challenging cases are still frequently reported. A study with 23 “case-control” dyads in which the “case” cats presented inappropriate *toileting* behaviour was conducted in order to investigate aspects potentially related to the problem, specifically: disease, stress, personality, emotionality, social behaviour. Among the participant “case” cats there were mostly females. About thirty-two percent of “toileters” (and 21.4% “controls”) had a medical problem interfering either with the urinary system or with the overall organism functioning. When alterations of the upper urinary system (with no obvious influence on urinary functioning) were considered, figures increased to the following: 46.4% (“toileters”) versus 35.7% (“controls”) presented medical alterations. No differences between “cases” and “controls” were seen with regard to faecal glucocorticoid metabolites and plasma dopamine. They also did not differ in the behaviours recorded and in terms of personality and social behaviour rated by the owners. However, they showed to be unreliable in their opinions towards several behavioural aspects of their cats’ personality. Results reiterate the importance of detailed medical check-ups in cases of inappropriate *toileting*, but also indicate a possible common factor, among these households, contributing to overall occurrence of medical illness.

Key-words: housesoiling, stress, dominance, personality, behaviour

4.1 INTRODUCTION

Domestic cats are well known for their fastidious nature. Their cleanliness habits specially concerning self-grooming and toilet use are notorious. Cats as young as one month old can manifest the typical scratching-burying elimination sequence without the need of specific training (BEAVER, 2005). It is suggested that the spontaneous occurrence of this behaviour does not depend upon previous observation of the mother either (BOWEN; HEATH, 2005). As their natural instincts prompt them to dig in soft materials and bury their waste, litterboxes are often the immediate and preferred choice even for the very young cat. In spite of this, inappropriate *toileting* behaviour in the form of urination and/or defecation outside the designated toilet area (typically the litterbox) is a common complaint by cat owners (VOITH, 1985). It constitutes the number one feline problem addressed by veterinary behaviourists (OLM; HOUP, 1988; BORCHELT; VOITH, 1986; MERTENS; DODMAN, 1996; NEILSON, 2004 b; BAMBERGER; HOUP, 2006).

Inappropriate urination encompasses most of the housesoiling cases: in the reported caseloads of both Olm and Houpt (1988) and Horwitz (1997) it represented 32% of the feline cases involving inappropriate *toileting* behaviours. Figures are even higher (reaching 60% - HORWITZ, 1997) when additional cases of cats that displayed both inappropriate urination and defecation are included. Given the unpleasant consequences for the owners and considering what the latter might expect from a scrupulously “clean” pet, inappropriate elimination constitutes the number one factor leading to cat relinquishment (PATRONEK et al., 1996; SALMAN et al., 2000) and a common reason for returning to shelter of a previously adopted cat (CASEY, VUNDEBUSSCHE, BRADSHAW, 2009).

In the home, cats presenting with inappropriate *toileting* behaviour, specially concerning urine deposits, tend to use a small number of alternative latrine sites generally composed of preferred soft surfaces such as carpet, linen, piles of clothes, bedding (NEILSON, 2004 a; BOWEN; HEATH, 2005). There are also litter aversions and litter preferences to consider (HORWITZ, 2002; BOWEN; HEATH, 2005,

HERRON, 2010) and a decision to use a given place to urinate might also be triggered by something going wrong with the litterbox per se (e.g. too small). The complete behavioural sequence of scratching and burying is commonly displayed, sometimes mimicked as a substrate to be manipulated is absent (MARDER, 1992). This might indicate a probable attempt to display a natural behaviour and indeed empty the bladder rather than simply soil the area.

Felys lybica, the wild ancestor of the domestic cat, like other felids, exhibits the same scratching-burying behavioural sequence for both urination and defecation, and commonly uses several toileting areas along their ranges (SCOTT, 1977). Domestic feral cats are commonly seen using communal latrines specially for defecation; urine deposits are found far from feeding stations, in multiple elimination areas and, on several occasions, are left uncovered (OLM; HOUP, 1988; FELDMAN, 1994). One might suggest that toilet arrangements provided by humans do not always work in favor of the cats' nature. Indeed, in a recent survey conducted in Brazil with 1137 cat owners, only 2.7% of them followed the rule of providing, one latrine per cat plus an extra one (DIAS, 2012). This is a general rule in cases of existence of inappropriate urination, but it can work well in help preventing the problem in multi-cat households. Besides, in 30% of the households litterboxes were located in the same location as where food was placed (DIAS, 2012).

Some problematic cats investigate but avoid the latrine thus urinating besides it or even walk away after investigation without using it. One could argue that in such cases cats behave as if "dissatisfied" with the latrine provided (COTTAM; DODMAN, 2007; HERRON, 2010). Other behaviours indicating a cat which is not content with its latrine are scratching the sides of the litterbox, scratching floor or a wall near the litterbox etc (COTAM; DODMAN, 2007). As it stands, and contrary to the owner's perception of these cats as dirty or spiteful, their refusal to use the proper latrine for urination seems to be the result of a challenge in the environment - the available latrine (or its related aspects - e.g: location, cleanliness status, containing substrate) may cause aversion or do not fit the preferences of that individual cat (LANDSBERG et al.; 1997; HORWITZ, 2002; NEILSON, 2004 a,b; BOWEN; HEATH, 2005, HERRON, 2010).

In the clinical setting, inappropriate *toileting* behaviour by domestic cats has long been differentiated from urine *spraying* (another “problematic” behaviour characterized by a cat’s use of urine to scent mark the environment) by its form (LANDSBERG, HUNTHAUSEN, ACKERMAN, 1997; HORWITZ, 2002; BEAVER, 2003; NEILSON, 2004 a,b; BOWEN; HEATH, 2005; HORWITZ; NEILSON, 2007; HERRON, 2010). For instance, *spraying* commonly involves small quantities of urine deposited on vertical surfaces (or on significant horizontal spots) with the cat commonly in a standing posture (HORWITZ, 2002; NEILSON, 2004 a). “*Sprayer*” cats generally keep use of the appropriate latrine for both urination and defecation (HORWITZ, 2002; NEILSON, 2004 a). By contrast, inappropriate *toileting* is mostly characterized by large amounts of urine on preferred horizontal surfaces with the cat in a squatting posture (HORWITZ, 2002; NEILSON, 2004 a,b). “*Toileter*” cats frequently give up using the latrine thus both urine and faeces are commonly found on inappropriate locations, although only one action may be affected (HORWITZ, 2002; NEILSON, 2004 a,b).

Differentiation between *spraying* and inappropriate *toileting* goes beyond their presentation. For a successful treatment, whenever possible, the identification of underlying causes of the behaviour (i.e. the cat’s motivation), which differs in the two forms of the behaviour, is recommended (LANDSBERG, HUNTHAUSEN, ACKERMAN, 1997; HORWITZ, 2002; BEAVER, 2003; NEILSON, 2004 a,b; BOWEN; HEATH, 2005; HORWITZ; NEILSON, 2007; HERRON, 2010). Whilst anxiety and distress are commonly listed as a major underlying cause of urine *spraying* (HORWITZ, 2002; NEILSON, 2004 a), inappropriate *toileting* is often related to litterbox management (HORWITZ, 2002, NEILSON, 2004).

In spite of several clinical behaviour guidelines proposing the aforementioned differentiation in order to achieve an accurate diagnosis leading to an effective treatment plan, several cases still remain as challenging (NEILSON, 2004 a). An important factor in this regard is the lack of ability of some owners to implement the therapeutic regimes recommended. This mainly involves making the latrine more attractive whilst making unattractive or unavailable the inappropriate target spots while avoiding punishment (for a review see HORWITZ, 2002; NEILSON 2004 a,b;

BOWEN; HEATH, 2005; HERRON, 2010). Even after the incorporation of the above treatment plan, some cats might still require re-training (OLM; HOUP, 1988), which adds extra work to an already difficult treatment. Another aspect which helps explain refractoriness in some cases is problem duration. As demonstrated in the study by Marder and Friedman (1998), in case of cats presenting the problem for a long time (i.e. more than one year), only 52% resolution was achieved (versus 100% resolution in the case of cats presenting the problem for one month or less). Indeed, since a great proportion of inappropriate *toileting* cases seen by behaviourists have long duration on average (i.e. 13 months in the study by HORWITZ, 1997), it helps explain, at least in part, why complete resolution of the problem is difficult to achieve.

Common assumptions made in relation to inappropriate *toileting* behaviour in domestic owned cats ought to be reviewed in order to better answer the question of why appropriate management regimes do not work in some cases. Some not so frequently mentioned factors such as cat personality, social behaviour and emotionality should be included in a more profound investigation. It has been suggested that fearful cats, especially in multi-cat houses, do not access the latrine areas due to their apprehensive state (BOWEN; HEATH, 2005). This being a result of either an unpleasant experience in the latrine or a social conflict arising from competition and leading to stressful reactions (OLM; HOUP, 1988; NEILSON, 2004 a). Even in the absence of empirical data to support the influence of such factors on inappropriate *toileting* behaviour, antidepressants and even “tender loving care” are recommended to help the apprehensive, sometimes frightened, soiling cat (OLM; HOUP, 1988; NEILSON, 2004; BOWEN; HEATH, 2005).

Finally, but not least, more care should be taken when considering the presence of concurrent diseases among inappropriate *toileting* cats. In spite of evidence of medical problems in these cats (HORWITZ, 1997; FRANK, ERB; HOUP, 1999) and the overall recommendation of a medical screening with physical examination and laboratory tests in order to eliminate medical causes in any case of inappropriate urination (LANDSBERG, HUNTHAUSEN, ACKERMAN, 1997; HORWITZ, 2002; BEAVER, 2003; NEILSON, 2004 a,b; BOWEN; HEATH, 2005;

HORWITZ; NEILSON, 2007; HERRON, 2010), the frequency with which medical check-ups are in fact conducted is not known. Furthermore, such advice is in need of an update as a more complete evaluation of the urinary system (e.g. via ultrasound or cystoscopy) may be necessary to reveal abnormalities that would not be seen otherwise (CHEW; BUFFINGTON, 2000).

This study aimed to perform an in-depth medical, physiological and behavioural evaluation of cats from multi-cat households presenting with inappropriate *toileting* behaviour. In order to identify factors associated with the cat (and not its environment) “control” cats from the same house were recruited thus constituting a case-control study (SCHLESSELMAN, 1982). The idea was to carefully investigate common assumptions as well as new aspects possibly featuring as underlying causes of the problem, specifically: diseases, stress, personality, emotionality and inter-cat social behaviour.

4.2 MATERIAL AND METHODS

As described in Material and Methods.

4.3 RESULTS

4.3.1 THE DYADS

The distribution of sex and breed among “case” and “control” cats was not significantly different (Exact Fisher test - sex: $p=0.371$; breed: $p=0.540$); “case” and “controls” also did not significantly differ as a function of their age (Student paired t-test, $p=0.286$). These are in accordance with our procedure of matching “case” and “controls” on the basis of these variables, whenever possible.

4.3.2 MEDICAL ASPECTS

Preliminary Exams

Preliminary exams, which determined selection of the dyads kept in the study revealed that out of 28 apparently healthy cats exhibiting inappropriate *toileting behaviour*, 9 (i.e. 32.1%) demonstrated an overt medical problem; as for their controls, this was found in 6 cats (out of 28 = 21.4%). The difference was not significant between “case” and “control” cats (Pearson Chi² test, $p=0.365$).

As to the excluded “case” cats, medical problems found were: renal insufficiency (3), leucocytosis (2), leucocytosis plus bladder plug (1), bladder lithiasis (1), bladder plug (1), hepatic disease (1). Excluded “control” cats had leukemia (1), bladder diverticulum (1), renal insufficiency (1), bladder lithiasis (1), abdominal liquid (1), hepatic disease plus urinary infection (1).

Even though selected cats were considered to be healthy on the basis of the initial health screen, this did not exclude some medical conditions which did not obviously impair either urinary or other organic system functioning. Four “case” and four “control” cats had one of the following alterations in the ultra-sound image of their kidneys: diverticulum calcification, heterogenous texture, decrease cortico-medullary definition, retraction and/or thinning of cortical area leading to loss of kidney definition. This meant that in total 13 out of the initial 28 recruited “toileters” (i.e. 46.4%) had medical abnormalities. In relation to the “controls”, 10 out of 28 (i.e. 35.7%) had alterations seen at the medical check-up. Among them, however, those with normal functioning were selected for the study together with the ones that had completely clear results.

Out of the 17 remaining dyads, 5 were removed from the study due to the presence of other cats in their households exhibiting urine *spraying*, thus leading to 12 selected “case-control” dyads selected.

A “control” cat showed urine excretion of high amounts of glucose. Blood levels of glucose as well as fructosamine were within normal reference ranges, though. Leucocytes count was high (i.e. 30,100 cells/mm³). Further urinalysis did not present glycosuria thus excluding any suspicion of diabetes mellitus.

Counts of blood leucocytes of “cases” and “controls” did not significantly differ (i.e. mean: “case” cats = 10,641.67 [SD = 2,191.91; n = 12]; “control” cats = 13,308.33 [SD = 7,206.87; n = 12]; Student paired t-test: p = 0.229). Serum creatinine as well as urea levels were also not significantly different when “toileters” were compared with their controls (i.e. creatinine - mean: “case” cats = 1.38 mg/dL [SD= 0.15; n=12]; “control” cats = 1.40 mg/dL [SD= 0.26; n=12]; urea - mean: “case” cats = 60.28 mg/dL [SD= 15.38; n=12]; “control” cats = 60.13 mg/dL [SD= 12.66; n=12]; Student paired t-test: p=0.848 [creatinine], p=0.968 [urea]).

Cystoscopy

Cystoscopy performed in six of the ten female cats with inappropriate *toileting* behaviour led to complete bladder visualization in only three them; in the remaining three cats urethra passage was very difficult and thus, given the risk injury, procedure was abandoned.

Whilst the bladder was healthy in two cats, in the third one it presented with submucosal petechiae (“glomerulations”), ulcerations and haemorrhage (figure 18) thus leading to a diagnosis of interstitial cystitis. Follow-up: on the days following cystoscopy the cat showed hematuria, polakiuria and painful urination (including high pitch vocalization before entering the litter box). Urine *spraying*, which had never been exhibited by the cat as well as a behaviour constituting of digging the front door of the house (followed by urination just there) arose. This cat was treated with amitriptyline (1mg/kg, BID) since first day after cystoscopy and for 90 days. Within a couple of weeks the cat returned to its previous pattern of eliminating large amounts of urine on inappropriate horizontal surfaces without signs of pain. The

owner reported, however, a decrease in frequency of inappropriate urination. The problem was still present after 6 months despite inclusion of behaviour therapy to further augment treatment.

Figure 18. Cystoscopic examination of a female “toileter” cat. Red arrow indicates petechiae and ulceration; white arrow indicates haemorrhage.

4.3.3 PHYSIOLOGICAL ASPECTS

Faecal glucocorticoid metabolites

The owner of one dyad (*Lua-Mila*) was unable to collect faeces of the cats as she never witnessed defecation of the participant cats. Results of the remaining 11 “case-control” dyads are therefore presented.

Whilst “toileter” cats had mean level of faecal glucocorticoid metabolites equal to 399.46 ng/g dry faeces (SD = 333.35; n=11), mean level of their controls was 369.46 ng/g dry faeces (SD = 283.17; n=11) (see figure 19). Shapiro-Wilk test revealed normality of the variable for both “case” and “control” cats ($p=0.365$ and $p=0.456$,

respectively). Faecal glucocorticoid metabolites for “case” and “control” cats did not show significant differences (Student paired t-test, $p=0.625$).

Figure 19. Faecal glucocorticoids metabolites for the 11 “case-control” TOILETING dyads.

Plasma bioamines

Blood samples of two “case” cats (*Stephania*, *Marie*) as well as their controls (*Pollux*, *Kiara*) were only sufficient to perform a biochemistry profile and complete blood count; bioamines analysis could not be performed. Results of the remaining 10 “case-control” dyads are thus presented.

Tubes test – Levels of serotonin in 7 shelter cats used for assessment of the validity of the method did not correlate when values derived from the same samples

stored in EDTA tubes were compared with the ones in dry tubes. Likewise, levels of epinephrine and norepinephrine (but not dopamine) did not correlate when values from EDTA tubes were compared with the ones in heparin tubes. Intra-class correlation coefficients (ICC) were as follows: serotonin (ICC=0.351, $p=0.180$); dopamine (ICC=0.781, $p=0.012$); epinephrine (ICC=0.147, $p=0.351$); norepinephrine (ICC=0.039, $p=0.454$). The more ICC is close to one, the more similar are the values from the two methods (i.e. two different tubes for samples storage). There is thus only significant similarity for dopamine values taken from samples stored in EDTA and heparinised tubes.

Duplicate samples test – Levels of serotonin and epinephrine (but not dopamine and norepinephrine) in the 5 owned cats did not correlate when values taken from duplicate eppendorfs derived from the same samples stored in EDTA tube were compared. Intra-class correlation coefficients (ICC) were as follows: serotonin (ICC=0.00, $p=0.915$); dopamine (ICC=0.714, $p=0.038$); epinephrine (ICC=0.00, $p=0.664$); norepinephrine (ICC=0.962, $p< 0.001$). The more ICC is close to one, the more similar are the values from the two eppendorfs (same EDTA stored sample). There is thus only significant similarity for dopamine and norepinephrine values taken in duplicate from EDTA tubes.

Plasma levels of serotonin, epinephrine, dopamine and norepinephrine are presented in figure 20.

Shapiro-Wilk test revealed dopamine normal distribution for both “case” and “control” cats ($p=0.234$ and $p=0.837$, respectively). Plasma levels of dopamine were not significantly different when “cases” and “controls” were compared (Student paired t-test, $p=0.562$).

Figure 20. Plasmatic bioamines for the 10 “case-control” TOILETING dyads.

	Serotonine (ng/ml)	Dopamine (pg/ml)	Epinephrine (pg/ml)	Norepinephrine (pg/ml)
“CASES”	N=10	N=10	N=10	N=10
<i>Mean</i>	33.26	179.07	170.77	340.00
<i>Median</i>	19.98	168.76	166.17	308.39
<i>Minimum-Maximum</i>	5.42-131.87	107.60-323.08	82.42-275.30	146.23-769.84
<i>Standard Deviation</i>	37.35	65.97	63.52	185.90
<i>Inter-quartile Range</i>	29.80	104.21	68.14	232.38
“CONTROLS”	N=10	N=10	N=10	N=10
<i>Mean</i>	85.80	192.52	229.44	385.69
<i>Median</i>	29.62	185.70	129.80	415.54
<i>Minimum-Maximum</i>	4.38-399.98	94.59-337.63	62.76-599.35	124.83-768.41
<i>Standard Deviation</i>	133.36	75.29	197,74	225.40
<i>Inter-quartile Range</i>	36.57	93.30	350.23	362.20

4.3.4 BEHAVIOURAL ASPECTS

Sociality

According to the owners’ opinion about their cats’ levels of *dominance* and *submissiveness* in relation to the other members of the group (both in a scale from 0 to 10, being 0=nothing and 10=totally), “case” and “control” cats did not significantly differ according to their *dominance scores* (Student paired t-test, $p=1.000$; mean level was 4.38 [SD=3.57] and 4.38 [SD= 3.69] - respectively for “cases” and “controls”, $n=12$ dyads) or to their *submissiveness scores* (Student paired t-test, $p=0.714$; mean level was 3.46 [SD=3.14] and 4.04 [SD= 3.04], respectively for “cases” and “controls”,

n=12 dyads). A *dominance index* composed of the dominance scores minus submission scores, from the same questionnaire-based scales, also did not show any significant difference when “case” and “control” cats were compared (Student paired t-test, $p=0.852$; mean=0.92 [SD= 6.24] and 0.33 [SD=6.02], respectively for “cases” and “controls”, n=12 dyads).

Regarding comparative analysis of the Dominance Trees derived from the agonistic interactions between the cats, reported by the owners, both *cat's rank* and *number of cats which it dominates* did not significantly differ between “case” and “control” cats (Wilcoxon signed-rank test, $p=0.531$, $p=0.906$, respectively, n=10 dyads). Whereas *inappropriate toileting* cats had median *tree rank* of 2.00 (IQR =1.00) and dominated a median of 0.00 individuals (IQR=1.00), their “controls” had a median *tree rank* of 2.00 (IQR=1.00) and dominated a median of 0.00 cats (IQR =1.00). As to overall *frequency of agonistic interactions* within the group, “sprayers” and “controls” had respectively median of 5.50 and 2.50 (“toileters” IQR=6.00; “controls” IQR=5.00; Wilcoxon signed-rank test, $p=0.500$, n=11 dyads).

As can be seen in the example of a Dominance Tree (figure 21) constructed for the *Brisa-Toquinho* household, the smaller the *cat's rank* the more dominant within the group the cat is. Cat's *rank* is given on the left with number of dominated individuals in parentheses. Dominance trees for the other households can be seen in Appendices P-Z.

Regarding comparative analysis of the Affiliative Trees derived from the affiliative interactions between the cats, reported by the owners, the *frequency of affiliative interactions* with any of the other members of the group did not significantly differ between “case” and “control” cats (Wilcoxon signed-rank test, $p=0.406$, n=11 dyads). Whereas *inappropriate toileting* cats had median frequency of 11.00 (IQR=28.00) their “controls” had median frequency of 13.00 (IQR=29.00). As can be seen in the example of an Affiliative Tree (figure 22) constructed for the *Brisa-Toquinho* household, the shorter the lines between the cats the stronger the affiliation between them. Such a distance, calculated from the reversion of their frequencies, are provided in the lines. Affiliative Trees for the other households can be seen in the Appendices P-Z.

Figure 21. Dominance Tree for the household containing the “case-control” dyad *Brisa-Toquinho* (dashed line indicates the “control” cat). Male cats are represented in blue and female cats in red.

Figure 22. Affiliative Tree for the household containing the “case-control” dyad *Brisa-Toquinho* (dashed line indicates the “control” cat). Male cats are represented in blue and female cats in red.

Personality

Out of the 11 owners (i.e. there were 12 dyads, but 11 households), only two did not complete the personality questionnaire for the participant cats. As for the reliability tests, in 8 households there was other family to answer the questionnaires.

Reliability of owners' opinions, measured through correlations between their scores and other family members' scores, revealed that there was significant similarity for all the variables: “timid” (intra-class correlation = 0.528, $p=0.013$), “bossy” (intra-class correlation = 0.671, $p=0.001$) “easy-going” (intra-class correlation = 0.502, $p= 0.018$). However, considering the inter-rater correlation suggested by

Meagher (i.e. at least 70%), scores for the three personalities were considered unreliable.

Whilst inappropriate *toileting* cats had mean scores of 5.45 for “bossy” (SD=3.60), of 3.45 for “timid” (SD=3.24) and of 7.50 (median) for “easy-going” (IQR=4.50), their “controls” had mean scores of 4.50 for “bossy” (SD=3.23), of 3.90 for “timid” (SD=2.97) and of 8.00 (median) for “easy-going” (IQR=4.00).

Regarding evaluation of reliability of owners’ opinion on 7 behavioural domains adapted from the work of Feaver, Mendl and Bateson (1986) (“active”, “curious”, “sociable with people”, “hostile to people”, “tense”, “fearful of people”, “equable with cats” - all in scales from 0 to 10, being 0=nothing and 10=totally), this was taken from comparisons between their opinions about the behaviour of cats in general taken in two moments: first whilst scoring about “case” cats and second while answering about “control” cats. A significant similarity was found for “sociable with people” (intra-class correlation=0.655, $p=0.011$), “hostile to people” (intra-class correlation=0.907, $p<0.001$) and “tense” (intra-class correlation=0.909, $p<0.001$) but not for “active” (intra-class correlation=0.058, $p=0.426$), “curious” (intra-class correlation=0.232, $p=0.235$), “fearful of people” (intra-class correlation=0.487, $p=0.056$) and “equable with cats” (intra-class correlation=0.058, $p=0.426$). However, applying the suggestion by Meagher (2009) also for intra-rater correlation (i.e. at least 70%), only the scores for “hostile to people” and “tense” could be considered reliable.

“Case” and “control” cats did not significantly differ in the two domains for which we gathered reliable score (Student paired t-test, $p=0.868$ [hostile to people], $p=0.856$ [tense]).

Mean scores rated by the owners for each of the 7 behavioural domains accompanied by the respective standard deviations, for “case” and “control” cats, can be seen in figure 23.

Figure 23. Owners' scores in the 7 behavioural domains for the 10 "case-control" TOILETING dyads.

	Active	Curious	Sociable with people	Hostile to people	Tense	Fearful to people	Equable with cats
"CASES"	N=10	N=10	N=10	N=10	N=10	N=10	N=10
<i>Mean</i>	0.05	-0.10	1.10	-2.40	-1.70	-2.40	-0.80
<i>Median</i>	0.00	0.00	0.00	-2.00	-1.50	-1.50	-2.50
<i>Minimum- Maximum</i>	-3.00-5.00	-5.00- 5.00	-4.00-9.00	-8.00-0.00	-6.00-4.00	-9.00-5.00	-4.00-4.00
<i>Standard Deviation</i>	2.45	2.96	4.15	2.37	2.79	4.50	3.43
<i>Inter- quartile Range</i>	3.00	4.00	4.00	2.00	4.00	6.00	7.00
"CONTROLS"	N=10	N=10	N=10	N=10	N=10	N=10	N=10
<i>Mean</i>	0.20	0.00	0.40	-2.30	-1.50	-1.60	0.70
<i>Median</i>	-0.50	0.00	-1.00	-2.50	-1.50	-2.00	2.00
<i>Minimum- Maximum</i>	-5.00-8.00	-4.00- 4.00	-4.00-9.00	-5.00-0.00	-4.00-3.00	-9.00-4.00	-5.00-4.00
<i>Standard Deviation</i>	3.88	2.58	4.33	1.77	2.01	3.69	3.06
<i>Inter- quartile Range</i>	4.00	4.00	3.00	3.00	2.00	2.00	2.00

Videos - Analysis of videos from 16 cats (i.e. 8 dyads) was performed. Videos from the four remaining dyads had to be excluded due to owners interference, at least with one of the cats, (e.g. taking the cat out of the hidden place, picking the cat onto the lap etc.), despite clear instructions not to do so.

First 3 minutes - most cats spent great proportion of the time in the hidden place ("cases"- median = 12.70s, IQR=106.80; "controls"- median= 68.70s,

IQR=92.80). There were not significant differences between “cases” and “controls” as to time spent neither hidden (Wilcoxon signed-rank test, $p=0.382$) nor in close proximity with the owner (i.e. Zone 1 + Zone 2 + under the owners’ chair = 50.40s [IQR=152.60] versus 37.20s [IQR=76.8]; Wilcoxon signed-rank test, $p=0.312$) or far from the owner (i.e. Zones 3 + Zone 4 + out of view = 50.70s [SD=46.41] versus 52.48s [DP=28.68]; Student paired t-test, $p=0.916$).

None of the cats stayed *on top of its carrier* and two (i.e. one “case” and one “control”) stayed *onto the owner’s lap*. Whilst only one “case” cat jumped into the *sink* and none onto the *table*, “control” cats did not jump either into the *sink* or onto the *table*.

About the *far from owner/(far + close) from owner* proportion, “case” cats did not significantly differ to the “controls” (“cases” - mean was 50.77%, SD=40.12; “controls” - mean was 37.85%, SD=33.82, Student paired t-test $p=0.566$) thus indicating that, “cases” and “controls” spent similar time distant (proportionally to close) from the owner.

As to *looking owner* and *investigating owner* behaviours, there were also no significant differences between “cases” and “controls” (Wilcoxon signed-rank test, $p=0.812$) considering the two together. Whilst “cases” spent a median of 4.20s (IQR=11.20), “controls” spent a median of 0.90s (IQR=13.20) on this composite behaviour. Significant differences were also not found when *looking owner* plus *investigating owner* behaviour were considered in terms of their frequency (“cases”: median=3.00 times, IQR=5.00; “controls”: median=1.00 times, IQR=5.50; Wilcoxon signed-rank test, $p=0.656$).

Investigate carrier was performed by 3 “cases” and 3 “controls”.

As to *meowing* which was only measured in terms of its frequency, “cases” and “controls” also did not significantly differ: whilst inappropriate *toileting* cats meowed a median of 3.50 times (IQR=16.00), their “controls” meowed a median of 0.50 times (IQR=10.00) (Wilcoxon signed-rank test, $p=0.437$).

Concerning time spent *stopped* versus *in movement*, whilst inappropriate *toileting* cats spent a median of 8.10s (IQR=10.00) moving and 73.15s (DP=63.80) stopped (in *sit*, *cowered* and/or *stand up* posture), their “controls” spent a median of

15.00 (IQR=22.20) moving and 36.38s (DP=38.10) stopped (in *sit*, *cower* and/or *stand up* postures) (Student paired t-test, $p=0.262$ [stopped]; Wilcoxon signed-rank test, $p=0.546$ [moving]).

About the *move/(move +stop)* proportion, “cases” and “controls” did not significantly differ: “cases” - median was 15.18%, IQR=47.85; “controls” - median was 50.06%, IQR=49.54, Wilcoxon signed-rank test, $p=0.156$) thus indicating that, “cases” and “controls” spent similar time moving (proportionally to stopped) around the room. *Moving overt frightened* which was measured and analysed in separate from general movement was only performed by 4 “case” cats.

The remaining 2 minutes (with food) - 2 “case” and 1 “control” cats ate the food provided. Whilst the two “cases” took on average 0,9s (median) to eat food, the “control” took about 1,8s. Food was eaten only once for all the cats.

4.4 DISCUSSION

Medical aspects

Significantly more females than males were recruited as *inappropriate toileting* “case” cats; among the 12 selected dyads, only two were composed of male “toileters”. Considering all recruited cats, there were males which were then excluded due to medical conditions identified during preliminary exams. Although this could lead to the argument of *inappropriate toileting* being mostly linked to disease in male cats, the fact that out of 34 initially recruited dyads only 9 were composed of male “toileters” might indicate that the problem, independently of its causative factors, seems to be more linked to the females. In some previous research a sex difference has not been identified (OLM; HOUP, 1988; NEILSON, 2004). But a previous caseload report found male cats overrepresented as to overall cases of *inappropriate elimination* (BAMBERGER; HOUP, 2006).

Marder & Engel (2002) working on inappropriate elimination (*spraying* excluded) found the selected cats were 59% females (versus 41% males). It is important to note that differently from the present study, in the mentioned research, cats which only defecated inappropriately were included (MARDER; ENGEL, 2002); according to BAMBERGER; HOUP (2006) males are overrepresented in this particular diagnosis. It seems that if a restricted diagnosis of inappropriate urination only (accepting that the cats will eventually defecate out of the box) is made (as in the present research, thus excluding the typical male modalities (i.e. exclusive inappropriate defecation and urine *spraying*) more female cats seem to remain.

Are female cats more prone to the negative effects of an inappropriate latrine? Further research are deserved in order to better understand this finding which could be simply due to our small sample size. If this is not the case, one hypothesis would be that, if an outlet is provided (such as an enclosed yard), some male cats might be going in search for outside alternative toilet spots which could pass unnoticed to the owner. Male cats have already given some evidence to be more proactive than females in their strategies when under undesirable conditions (e.g. separation from the owners: whilst males vocalized more, females exhibited self licking) (SCHWARTZ, 2002).

No significant differences between “case” and “control” cats as to the occurrence of medical conditions were found. These results are surprising as inappropriate *toileting* is traditionally considered to be associated with disease (HORWITZ, 2002; BOWEN & HEATH, 2005, NEILSON, 2004, HERRON, 2010), we did not expect the control cats, apparently healthy and without exhibition of any overt behavioural problem, to be equally ill. Importantly, percentage of cats presenting a medical condition (i.e. 32.1%) among the ones engaging in inappropriate *toileting* behaviour corroborates previous research which has indicated a similar prevalence of lifetime history of urinary tract disease among “toileter” cats (OLM; HOUP, 1988; HORWITZ, 1997). However, a 21.4% prevalence of medical problems among “control” cats is very unexpected; a previous report of 100 apparently clinically healthy cats revealed that only 6% only had medical problems (e.g.

hyperthyroidism, urinary infection) (GARCIA;BRUYETTE apud LANDSBERG; WILSON, 2005).

One possible explanation for such a result probably lies in environmental characteristics (or even ownership style) predisposing diseases in both “case” and “control” cats. Depending upon combinations of external factors (e.g. decrease in water availability, provision of a low quality diet) and individual predispositions, medical problems interfering with the urinary system may arise, thus likely leading to inappropriate urination in part of the sample. In other cases, non-urinary illness may develop and likely result in other medical signs which may not be perceived by the owner of multiple-cats, at least in its early stages. The hypothesis is that an appropriate care may not be provided to the participant cats and this may also include latrine inappropriateness. Whether this is a problem of the households in the study only, or if it represents a characteristic in overall cases of inappropriate *toileting* behaviour remains to be explored.

Selection criteria in this study were strictly controlled with regard to medical components. Nonetheless, supposedly “healthy” selected cats presented medical conditions which could constitute either co-morbid or linked to inappropriate *toileting* behaviour. For instance, “cases” and “controls” had subtle renal alterations seen during imaging examination such as increased echogenicity, calcified diverticulum, lost of cortical-medullary definitions, cortical thinning. Since signs of renal malfunctioning (seen by a combined interpretation of their urinalysis and serum creatinine levels) were absent, these cats were kept in the study. At least in their cases, and at that moment in time, renal alterations were considered unlikely to be interfering with urination behaviour. One may argue, however, that on the basis of these findings such cats should be considered as having chronic kidney failure (CKD/stage 1 - classification system by the International Renal Interest Society - IRIS, mentioned by ROUDEBUSHI et al, 2009; WAKI et al, 2009). In any case, their clear urinalysis and creatinine/urea levels within normal reference range reinforce our argument in favour of normal urinary functioning.

Our finding concerning renal alterations may be simply incidental due to our small sample size. On the other hand, it may pose some questions on whether

inappropriate *toileting* behaviour may be a precocious sign of renal malfunctioning. Renal calculi, as another example of renal alteration, were also detected in a previous study including radiography of the urinary system as part of the medical check-ups for cats presenting with urine *spraying* (FRANK, ERB; HOUP, 1999). Based on the present results, and despite the suggestion by some to focus on the lower urinary tract (OLM; HOUP, 1988; NEILSON, 2004 a,b; HERRON, 2010) and perform urinalysis only (HORWITZ, 1997; MARDER; ENGEL, 2002) to rule out medical problems in cases of inappropriate *toileting*, emphasis should be made on the need for a complete medical check-up, including upper urinary tract imaging and biochemistry profile, for all cases of inappropriate *toileting* behaviour.

Diagnosis of interstitial cystitis in one “toileter” female cat was particularly interesting. However, given that the bladder was completely inspected in only three out of six females, we cannot exclude the possibility of other female cats having similar alterations. Pathognomonic submucosal petechiae (i.e. “glomerulations”) (CHEW; BUFFINGTON, 2000; HOSTUTLER, CHEW; DIBARTOLLA, 2005) together with bladder haemorrhage were considered for the definite diagnosis. As suggested by Chew and Buffinton (2000) without cystoscopy, several cases of inappropriate urination are at risk of being erroneously diagnosed as a purely behavioural problem. Indeed, in the case of this female cat, the normal results in all the other previous exams together with the lack of clinical signs, had led us to diagnosis of an exclusive behavioural condition. It is important to mention that the owner had dedicatedly tried different therapeutics in the past years with the problem always recurring, possibly due to an inaccurate diagnosis.

The suggestion by some authors that submucosal petechiae are nonspecific (RECHE-JUNIOR; HAGIWARA, 2001, 2005) and could be even a result of the cystoscopic procedure itself (JOHANSSON; FALL, 1990) deserves further discussion. As suggested by RECHE-JUNIOR; HAGIWARA (2001, 2005) biopsy and histopathological exam would be necessary in order for a definitive diagnosis to be made. Even the haemorrhage has been pointed by some as a complication of the procedure itself (MESSER, CHEW; MCLOUGHLIN, 2005). In our case, as other possible causes of petechiae (e.g. infection, neoplasia) had been previously excluded

by other exams, we believe alterations were probably compatible with interstitial cystitis.

The possibility of an iatrogenic cystitis resulting from the procedure cannot be completely ruled out, but the identification of petechiae right when the endoscope reached the bladder seem to indicate otherwise. It is important to reiterate that the occurrence of polyuria, palakiuria and even hematuria for some days after cystoscopy is expected (MCCARTHY, 2005; TAMS; RAWLINGS, 2011) and thus do not indicate necessarily that the abnormalities seen are injuries caused by the procedure itself.

It ought to be considered, however, that cystoscopy was performed some time after the other exams and so we cannot know whether alterations would be seen on those exams if they were performed on the day of cystoscopy. As described by Neilson (2004 a) feline lower urinary tract diseases can progress as a “waxing and waning” processes, thus making clinical signs as well as laboratory and imaging findings transient. Cystoscopy is thus a very appropriate investigation tool as compatible bladder alterations are seen even in patients without signs of the disease (RECHE-JUNIOR; HAGIWARA, 2001).

Additional concerns relating to procedural risks and post-cystoscopy complications, however, deserve further attention. Despite the accurate diagnosis, a worsening of the problem followed the procedure with the cat even exhibiting urine *spraying* (probably as a result of pain) was seen. Importantly, however, medication was introduced as a key part of the therapeutic regime for interstitial cystitis. If the diagnosis had not been made, the case would carry on being considered as a solely behavioural inappropriate *toiletting* behaviour which would exclude the use of psychotropics. It could be that longer term psychotropic use (another one targeting serotonergic system) in association with more specific behaviour modification (SEAWRIGHT et al., 2008) may lead to a better outcome in the present case.

Physiological aspects

As to faecal glucocorticoid metabolites, as expected, no significant differences between “cases” and “controls” were seen. With the exception of very sensitive cats reacting distressfully to an inconvenient latrine, cats engaging in inappropriate *toileting* behaviour are not commonly supposed to be distressed (HORWITZ, 2002; NEILSON, 2004 b; BOWEN; HEATH, 2005; HERRON, 2010).

It may be that our “toileter” cats are as relaxed as their controls; conversely, they could be as stressed as the “non-toileters”. Evidence for the latter explanation exist and come from a comparison to previously published research with laboratory cats whose faecal glucocorticoids levels (measured by the same EIA-oxoacetylcholonalone) were lower to the ones presented here (PALME, SCHATZ; MOSTL, 2001; SCHATZ; PALME, 2001). For instance, whilst their median baseline levels were 196 ng/g (SCHATZ; PALME, 2001), ours were 355.91 ng/g (values considering both “case” and “control” cats). It ought to be considered that our extraction method used a more concentrated alcohol (90% methanol as opposed to their 80% methanol – SCHATZ; PALME, 2001; PALME, SCHATZ; MOSTL, 2001) which was thus expected to result in more glucocorticoid been extracted – the increment was predicted to be about 5% only, though.⁴

In a recent unpublished study by our research team, faecal glucocorticoids metabolites from domestic owned cats (n=57) coming from multi-cat houses of 3-4 cats (n=17) were measured. Median levels found through a 90% methanol extraction followed by EIA-oxoacetylcholonalone dosages were 227.11 ng/g. Overall, as endocrinological results stand, households in this study were probably stressful environments (cats were likely aroused, probably stressed). Extreme glucocorticoid levels were found in some residences (e.g. “stephania-pollux” and “darah-thor” dyads are from the same household). It is possible that these are sensitive cats overreacting to inappropriate aspects of their environment, which could potentially include their latrine, among others such as decrease available space.

One may argue that the exclusive measurement of faecal glucocorticoids is not an appropriate tool to investigate stress. Indeed, given the lack of a reference range, our

⁴ Verbal information provided by Prof.Rupert Palme in November/2011 at Viena University of Veterinary Medicine.

conclusions would be rather limited if we could not compare our results with other studies. Another limitation regards the hypothalamic-pituitary-adrenal HPA axis being attenuated under prolonged stress thus not being the ideal tool for measuring chronic stress (KEAY et al., 2006; MORMED et al., 2007). As stated by Beerda et al. (1999), increased cortisol levels are a strong indication of chronic stress, although the lack of it may not exclude possibly stressed animals.

As will be seen later, differences in the behaviour of “toileter” cats (in comparison with their controls) during first visits to the veterinary hospital were not identified, thus supporting our argument that cats engaging in inappropriate *toileting* behaviour are not differently emotionally distressed. Glycosuria and leucocytosis seen in the exams of one “control” cat was probably an overreaction to acute stressors (e.g. examinations, collection of blood and urine) from a cat already stressed at home.

HPLC method for measuring feline plasma bioamines from samples stored in EDTA tubes (revealed by both *tubes test* and *duplicate samples* tests) demonstrated unreliability for most of the neurotransmitter (the exception was dopamine). This strongly restricts our understanding about what is going on regarding emotionality of “toileter” cats. If a reliable measurement had been achieved, neurochemical aspects might help to confirmed our finding that these cats are equally aroused, and possibly in the same emotional state. Dopamine levels at least, in our results support this hypothesis.

A previous study with the purpose of correlating behaviour problems and emotionality of pet dogs used EDTA tubes to store blood samples subsequently submitted to HPLC for measuring serotonin and catecholamines (RIVA et al., 2008). Indeed, at least regarding serotonin measurement, some still recommend EDTA storage and natural decantation at the refrigerator when collecting plasma (TEKES, 2008). Even though the same procedure was recommended to us and undertaken in the present research, further knowledge on our part that such a procedure was not ideal, led us to undertake the reliability tests in order to clarify whether our results were reliable and of any use. Unfortunately, at that point in time, collections from the participant cats had all been completed.

Indeed, studies on stability of catecholamines, for instance, recommend that heparinized tubes containing an antioxidant and immediate centrifugation are preferred (BOOMSMA et al., 1993; DÁLESANDRO et al., 1990). Even though our samples did not wait for longer than 1h before centrifugation, and eppendorfs were kept at -80C (these are all recommended procedures), tubes were sometimes kept at 4C before undergoing centrifugation, following the protocol by TEKES (2008). According to BOOMSMA et al. (1993) at 4C blood cells may break down thus releasing catecholamines and increasing values in its dosages.

Such an effect, however, would not negatively affect our study, whose aim was to use the comparison “cases” versus “controls”; since samples from both cats were collected and stored at the same time, an increasing effect would affect both samples anyway (i.e. results would be reliable but not valid). The same line of argument could be used to address the problem of some of our samples being stored for over a year; in spite of the 12 months storage limit recommended by Boomsma et al. (1993), i.e. any expected decay would affect equally samples from both cases and controls. However a more critical problem was that the appropriate tubes were not used. Antioxidative properties of EDTA did not seem to be sufficiently good to prevent a previously reported chaotic oxidative process (BOOOSMA et al., 1993) thus resulting in unreliable measures seen in the *duplicate samples* test.

Our investigation of reliability is very important to prevent incorrect inferences (due to unreliable measures) in future studies. A recently published paper about the effects of drug detection training on dog reactivity used antioxidant containing tubes to store samples later analysed for catecholamines and serotonin (RIVA et al., 2012). This research was conducted by the same group which back in 2008 used EDTA with no antioxidant tubes for storing blood samples (RIVA et al., 2008). Curiously, in the recent paper (RIVA et al., 2012) nothing was mentioned about either the reliability of the measures in the former research (RIVA et al., 2008) or why they changed their methodology.

Behavioural aspects

According to the owner reports, “cases” and “controls” did not significantly differ in their dominance and submission scores. One may argue that owners’ opinion are of limited value, or even criticize its use as being a non-scientific method (for a review, see MEAGHER, 2009). Indeed, evidence of reliability and validity properties of their opinions are lacking and they might indeed not reflect reality. However, at least in terms of social behaviour, their opinions are in accordance with results from agonistic matrices (which also derived from their observations), thus adding some validity to their scores.

“Case” and “control” cats were equally involved in agonistic interactions (e.g. biting, hissing, growling, and attacking) within the group. Besides, no consistent social position at the Dominance Trees was seen for the “toileters”. For the construction of these, aspects of linear dominance hierarchies such as if cat A dominates B, and B dominates C, then A also dominates C, is assumed (IZAR; SATO, 1997; FURNARI, 2011). Linear dominance hierarchies have been previously identified in studies on the social organization of groups of domestic feral cats (NATOLI; DEVITO, 2001; NATOLI, BAGGION; PONTIER, 2001) and so the use of it in this study, as an attempt to detect social patterns among “toileter” cats, should not be seen as data over interpretation.

In the present study, “toileters” were sometimes high in the dominance hierarchy, sometimes at the bottom of it, or in between the two extremes. Number of dominated individuals also did not significantly differ between “cases” and “controls”. Results corroborate the assumption that inappropriate toileting, differently from urine *spraying*, is not considered to be associated with social status (HORWITZ, 2002, NEILSON, 2004 a,b, HERRON, 2010). The report of some cats avoiding the use of the litterbox (and thus eliminating inappropriately) as a result of a probable fear of conflicts with others (NEILSON, 2004, BOWEN; HEATH, 2005) seems to reflect isolated cases rather than being the general rule.

Regarding affiliations, “toileters” were sometimes very close to other cats in some houses, yet distant from the group in others. In one situation only, the “case” cat was completely isolated from the group. Frequency of affiliative interaction between “cases” (versus “controls”) and other cats in the group also did not differ

significantly. As social hierarchies can also be constructed on the basis of social bonding and some have suggested that the dominant cat is the one receiving higher amounts of amicable behaviour (MACDONALD, 1987; NATOLI, BAGIO; PONTIER, 2001; CAFAZZO; NATOLI, 2009), our results for affiliation corroborates the ones for agonism thus confirming that inappropriate *toileting* behaviour is not linked to social dominance. Likewise, it reinforces our previous conclusion that inappropriate *toileting* is not a behaviour exhibited by isolated submissive cats avoiding use of the latrine to prevent encounters.

In terms of personality, inter-rater reliability test based on a comparison of the owners answers with those from another family member revealed statistically significant similarity. However, MEAGHER (2009) suggests that correlations should be of 0.7 or greater to be really considered significant; according to this, and taking the three questions on behavioural styles (i.e. “bossy”, “timid”, “easy-going”), we can conclude owners were not reliable. Family members did not strongly agree on how their cats fit with those styles. As it is not possible to know whether one or the other, if any, conform with reality, none of the opinions can be reliably taken into consideration.

A similar result was found with regard to the scores for the seven behavioural domains adapted from the work of Feaver, Mendl and Bateson (1986). Significant differences were not found when scores for “cases and “controls” were compared; intra-observer reliability test was strong (i.e. > 0.7 – according to MEAGHER, 2009) for “sociable with people”, “hostile to people” and “tense”, but not for “active”, “curious”, “fearful of people”, and “equable with cats”. Despite the definitions provided, they may not have understood the question. It may be that the question about “cats in general”, as part of all the questions regarding “their cats”, may have led them to choose different (and inappropriate) strategies when scoring (e.g. they may have answered about their own cats at both instances, being the second time in relation to “cats in general”). Whatever happened, their ratings for their own cats on “active”, “curious”, “fearful of people” and “equable with cats” cannot be considered further. As reaction to people and tenseness, “toileters” did not differ from their

controls, again suggesting that the difference between “toileters” and controls does not lie in their definable personality.

Some may argue that other more objective temperament test such as the Feline Temperament Profile (FTP) used in previously published research on domestic cat individuality (SIEGFORD, 2004; IKI et al., 2011) could have been used instead. However, considering the extra time which would be spent in the already long visit to the veterinary hospital, along with the fact that cats change behavioural strategy across specific contexts (KIDDIE; CASEY, 2009), an integrative evaluation of more general patterns based on everyday behaviours rated by people familiar to them seemed to us much more valuable (FEAVER, MENDL; BATESON, 1986; MEAGHER, 2009).

“Cases” and “controls” did not significantly differ in the behaviours seen in the videos. They all spent a long time in the hiding place and when not there, they seemed to stay slightly more time in static body postures (i.e. not moving). Food was eaten by only a few cats. This seems to represent well the feline attitude: when under stressful conditions they mostly show inhibition and inactivity.

In conclusion, the cats did not differ in any of the investigated aspects. The occurrence of medical problems among “cases” and “controls” cats deserves further investigation as it might indicate a management problem common to multi-cat households in which some of the cats exhibit inappropriate *toileting*. This would corroborate the traditionally proposed link between the inappropriate toileting behaviour and inappropriate litterbox management (NEILSON, 2002 a,b; HORWITZ, 2002, HORWITZ; NEILSON, 2007; BOWN; HEATH, 2005, HERRON, 2010).

Discussion & Conclusions

“Integrating Sprayers and Toileters”

5.1 Feline Inappropriate urination: past, present and future

Inappropriate urination is a problem with serious implications for both cats and their human families. Early reports about attempts to solve the problem by neurosurgery show how seriously a problem associated with a normal behaviour can be for the human-cat relationship. Such invasive interventions (e.g. medial preoptic-anterior hypothalamic lesions, olfactory tractotomy) were recommended for use on problematic cats to avoid euthanasia (HART; VOITH, 1978, HART, 1982). The use of long-acting progestins (HART, 1980) was perhaps a more realistic therapeutic approach for urine *spraying*, although they are associated with severe side effects. Later more effective psychotropic drugs with less complications such as diazepam and buspirone, and most recently fluoxetine and clomipramine, have further improved the treatment options for urine *spraying* (COOPER; HART, 1992; HART et al., 1993; PRYOR et al., 2001; LANDSBERG; WILSON, 2005).

Despite the scientific and pharmaceutical progress in the last decades, still nowadays inappropriate urination in general still constitutes a problem leading to feline relinquishment and is the most frequent behavioural problem seen by specialists (SALMAN et al., 2000; APBC, 2005). Even in a country such as Brazil where veterinary behaviour medicine is in its infancy, feline inappropriate urination is the most frequent behavioural problem seen by veterinarians (SOUZA-DANTAS et al., 2009), with the problem not only recognized but also the help of veterinarians sought for assistance in managing it (SOUZA-DANTAS et al., 2009). Availability of different sorts of over-the-counter products (COTTAM; DODMAN, 2007), in particular the synthetic pheromones (MILLS, 2005; MILLS, REDGATE; LANDSBERG, 2011), has possibly contributed to the recent decline in feline inappropriate elimination cases seen by behaviourists (BAMBERGER; HOUP, 2006). However, for general veterinarians reports of these cases remain common (BERGMAN et al., 2002; SOUZA-DANTAS et al., 2009). In spite of the available protocols for accurate diagnosis and clear-cut therapeutics (LANDSBERG et al., 2007; HORWITZ, 2002, NEILSON, 2004; BOWEN; HEATH, 2005; HERRON, 2010),

complete resolution appears rather hard to achieve. Among the reasons for this are the lack of full owner compliance (CASEY; BRADSHAW, 2008; MARDER; ENGEL, 2002) and inappropriate treatment regimens (BERGMAN et al., 2002). Difficult to implement therapeutics also play a role; however, as shown by Marder and Engel (2002), if presented in a clear manner, behavior modification at least is considered feasible by most owners. However, when confinement and oral medication is required, commitment is likely to decrease (MARDER; ENGEL, 2002).

Beyond the above mentioned limitations, several anecdotal reports about refractory and atypical cases present the question “why is success so hard to achieve?” Possible flaws in the treatment process concern either the differentiation of the diagnosis (i.e. urine *spraying* versus inappropriate *toileting*) or the commonly implemented therapeutic regime. For instance, cleanliness routine and number of litterboxes were close to ideal in most households of “toileters”; the synthetic pheromone Feliway (indeed indicated for treating urine *spraying* – for a review see MILLS, 2005) had been tried in at least one household of “sprayers”. But whether these treatments had been correctly implemented is certainly open to question (e.g. are litterboxes located at the right places? was Feliway kept constantly plugged in?). Whatever the answer, persistence of incorrect interpretations (e.g. “the cat is take out its revenge”, “the cat is dirty”) and inappropriate actions (e.g. the use of reprimands) on the owner’s behalf are likely to negatively affect any therapeutic regime. As seen in this research, for both *spraying* and inappropriate *toileting* cases, owners were mostly mistaken about the reasons why their cats did the behaviour and took inappropriate actions: the vast majority of them used punishment.

Central to success is veterinary training and client education. General practitioners are not fully prepared for dealing with these cases (BERGMAN et al., 2002; SOUZA-DANTAS et al., 2009), in spite of feeling confident to do so (PATRONEK ; DODMAN, 1999); furthermore, referral of cases to specialists does not always occur (FATJO et al., 2006; SOUZA-DANTAS et al., 2009). Owners of soiling cats need clarification on why their cats manifest the behaviour and the best approach to help them. As proposed by Landsberg, Hunthausen and Ackerman, (1997) clear explanations provided to the owners are a critical part of veterinary

behavioural counselling. Without dissemination of good research, valuable information will continue to be kept on the hands of specialists only, and owners will still end up abandoning their animals in the light of lack of knowledge about feline nature (PATRONEK et al., 1996).

5.2 About the problem and the problematic cats: is there something new?

Male “sprayers” and female “toileters” were the vast majority of our “case” participant cats. Indeed, when the two were compared in terms of sex, there were significantly more males among “sprayers” and more females among “toileters” (Exact Fisher test test, $p=0.002$). Concerning *spraying*, such a result corroborates the traditional assumption that scent marking is mostly exhibited by male cats (FELDMAN, 1994). However, regarding inappropriate *toileting*, our finding is new, albeit not surprising, as previous research indicated males were overrepresented actually considered a more general diagnosis (BAMBERGER; HOUP, 2006). As it seems, it may be that when considering a more restricted diagnosis in which urine *spraying* and exclusively defecation outside the litterbox are excluded (the two modalities in which male cats are overrepresented) more females remained (MARDER; ENGEL, 2002; BAMBERGER; HOUP, 2006). Besides our small sample size, our finding was probably due to the fact that, different from others, we narrowed the diagnosis to cases of exclusively inappropriate urination.

When “sprayers” and “toileters” were compared in terms of breed, no significant differences were found (Fisher exact test, $p= 0.167$). Previous studies have showed more Persians among inappropriate *toileting* cases (BEAVER, 1989; BAMBERGER; HOUP, 2006) with no breed predisposition for “spraying” (BAMBERGER; HOUP, 2006). BEAVER (2005) suggested an impaired ability to learn proper latrine behaviours in Persian cats. In the present research, a small sample size may be among the reasons why similar result was not found. Persians are a common cat breed in Brazil; in an unpublished result by our research team with multi-cat households

drawn from the general population, Persians represented about 30% of the pure-breed cats (among the 120 participant cats, 19% were pure-bred cats).

Concerning age of the cats, no difference was found between groups (1: all cats from *spraying* households versus 2: all cats from *toileting* households). (Two samples t-test, $p=0.111$). One previous study found a similar median age of problem evaluation in cats presenting with various behavioural problems (including inappropriate *toileting*) (i.e. 5.5 years) (BAMBERGER; HOUP, 2006). The fact that in both groups of the present research owners reported long time presence of the problem (i.e. 1 year at least) suggests action should be taken in this matter as well. The longer the problem is present the worse its prognosis (MARDER; FRIEDMAN, 1998). Advice concerning the importance of taking action as soon as problem starts should be added to the veterinary continuing education and public outreach programs. For instance, questioning regarding feline elimination behaviour could be incorporated into the routine consultation.

Our finding about the long duration of the problem reported by the owners indicates that cat owners reasonably tolerate this problem in the cat. It is also consistent with a previous finding that before deciding to give up on a problematic cat, owners generally had lived with it for about 1-2 years (unlike dogs whose owners abandoned the animal much earlier, SALMAN et al., 2000). In Brazil, this delay may be longer as euthanasia or abandonment to a shelter is not an easy option (SOUZA-DANTAS et al., 2009). However, this means there is time to educate owners and to get their agreement to treatment; long time frustration, however, may lead to resignation and/or lack of will, so veterinarians must be prepared to deal with these negative aspects too.

5.3 Task Force: General practice + Clinical ethology

A very relevant aspect of the present research was the prevalence of medical conditions. These were detected with similar frequency in both apparently healthy

“sprayers” and “toileters” (Pearson Chi² test, $p = 0.639$). Despite recommendations to focus on ruling out medical problems in cases of inappropriate *toileting* only (TYNES et al., 2005), our findings emphasize that a medical work-up should be undertaken in any case of inappropriate urination as previously suggested (e.g. FRANK, ERB; HOUP, 1999)

Medical alterations in the selected cats (both “sprayers” and “toileters”) were common and deserve further discussion. Investigations of the link between early renal disease and different types of urinary act, particularly when urinary function does not seem to be diminished are lacking. Likewise, the potential of renal formations (e.g. calculi within the kidney) to cause pain and as consequence change urination behaviour has been completely overlooked. Thus, if such conditions act either as causes or contributors to inappropriate urination (particularly in the *spraying* cases) and have not been detected due to medical exams being focused on the lower urinary tract (OLM; HOUP, 1988; NEILSON, 2004; HERRON, 2010) or restricted to urinalysis (HORWITZ, 1997; TYNES et al., 2005), this might explain, at least in part, the refractoriness of some cases: since part of the problem has not been recognised.

Since the veterinary goal is to provide complete care for the feline patient with a urinary behaviour problem (NEILSON, 2004 a,b; HERRON, 2010), a detailed medical examination including imaging evaluation of the upper urinary tract should be encouraged. Whether alterations found are considered cause, consequence or just co-morbid entities remains unclear. Therapeutics for some conditions (i.e. CKD-stage 1, renal calculi, renal cyst) will not go far beyond monitoring anyway as it is still unclear (ROSS et al., 2007), but a relapse in urination in the light of medical progression, or an improvement as a result of medical treatment, would help explain why the case had not achieved success despite the appropriate behaviour treatment.

As to interstitial cystitis, the detection of the disease via cystoscopy provides an accurate diagnosis and would indicate that a more multimodal approach is required (CHEW et al., 1998; WESTROPP; BUFFINGTON, 2004; GUNN-MORE; CAMERON, 2004; GUNN-MORE; SHENOY, 2006; BUFFINGTON et al., 2006; SEAWRIGHT et al., 2008). But should we start recommending cystoscopy to all cases of inappropriate

urination? Given the need and risks of anaesthesia, possible complications, cost, and mainly the fact that full inspection of the bladder is not possible in all cases, our suggestion is that cystoscopy should be left for refractory, atypical and more obscure cases. Cystoscopy could only be completed in half of the submitted females. It may be the case that, as proposed by Chew and Buffington (2000), as many as 60% of cats exhibiting the sole inappropriate *toileting* behaviour will have cystitis detected at cystoscopy; however, for several reasons (e.g. cost, lack of licensed veterinarians), at least in Brazil, cystoscopy is not yet a realistic option for the average cat owner nor for the general practitioner.

Before its execution, owner must be made aware of the procedure itself and the risk of temporarily worsening of the problem, as appeared to occur in two cases in this study. Furthermore, it is essential to explain that urethral passage by the endoscope may not be possible, in which case the procedure will be abandoned without a diagnosis. Inability to enter the urethra is the most common complication of cystoscopy (MESSER, CHEW; MCLOUGHLIN, 2005). Further studies on the conditions required for a successful cystoscopy are therefore required; among the females in which endoscope did not reach the bladder were some large animals (over 5,0 Kg), thus size should not be a major consideration for deciding whether or not to undergo cystoscopy. Although it seems to be simple, cystoscopy requires a highly trained team (MESSER, CHEW; MCLOUGHLIN, 2005; MCCARTHY, 2005); for us, the procedure seemed rather difficult.

Whilst in *spraying* households medical conditions were more common in “sprayers” than in “controls”, in *toileting* households “toileters” and “controls” were equally affected by medical problems. As it stands, *toileting* and *spraying* households probably differed in some aspects which might be linked to illness predisposition in the former. This is the first time medical problems are identified at a high frequency not only among apparently healthy “toileter” cats but also in other cats sharing the same environment with them, thus indicating a common external problem in these houses. This gives support to the traditional argument of inappropriate *toileting* as a behaviour problem linked to poor management (e.g. unacceptable cleanliness of the litterbox, inappropriate distribution of the litterboxes etc) (LANDSBERG,

HUNTHAUSEN; ACKERMAN, 1997, NEILSON, 2004; HORWITZ, 2002, BOWEN; HEATH, 2005, HERRON, 2010). Inappropriate household procedures may be at the root of both medical and behavioural problems seen in these households.

More investigation of this matter is therefore required. If the hypothesis concerning the link between the same management and disease as well as inappropriate *toileting* is true, a broader enquiry on overall environmental management and feline care should be made when the clinician is dealing with *toileting* problem in multi-cat houses. In this sense, general practices of good feline care should be also included to the behaviour therapeutics.

5.4 Unbalanced emotions: are we barking up the right tree?

Differences between “case” and “control” cats as to faecal glucocorticoids metabolites were not found in neither of the groups (i.e. *spraying* and *toileting*). But levels in group 1 (*spraying*) was significantly greater than group 2 (inappropriate *toileting*) (Kruskall-Wallis test, $p=0.026$). Normal reference ranges (i.e. expected values for non-stressed cats) if possible has yet to be established for the species. However, a direct comparison between the results obtained here with those found by Schatz and Palme (2001), Palme et al. (2001), and with an unpublished research from our team (RAMOS et al., unpublished) is possible and confirms the higher values in the present research; cats in “*spraying*” households are overall more aroused, and probably distressed.

The question which then arises is: “if equally aroused (equally distressed), why do only some exhibit urine *spraying*?” The answer to this question is not obvious and gives rise to several hypotheses. First of all, our finding suggests that *spraying* is unlikely to be a consistent stress sign as not all aroused cats appear to react with *spraying*. Conversely, *spraying* could be a sign of stress management; in other words, it could represent an animal that has found its way to deal with a stressful condition. For instance, it might be that glucocorticoids levels in *spraying* cats could be even

higher before *spraying* started, with a decrease happening to the currently seen levels. However, considering that these cats were engaged in the behaviour for so long, this hypothesis seems unlikely (a greater decrease would be expected if *spraying* was a successful strategy). Another possibility is that *spraying* is an unsuccessful attempt to get stress relief.

Behavioural strategies aiming at managing an aversive situation and capable of either reducing physiological measures of stress or removing the aversion itself are also known as coping behaviours (WECHSLER, 1995). For instance, *hiding* in the form of resting behind the litter pan was found to be negatively associated with urinary cortisol in caged laboratory cats (CARLSTEAD, BROWN; STRAWN, 1993). In this sense, *hiding* can be considered a coping strategy for confined laboratory cats. *Spraying*, however, did not show the same effect for multi-cat households, as seen in the present research. Thus, it might be argued that *spraying* could be seen as a stereotypic behaviour, for some the end point of an unsuccessful attempt at coping for a long period (WESCHSLER, 1995). Whilst stereotypes can still reduce stress, evidences of this are not unequivocal (WESCHSLER, 1995).

Our finding in relation to faecal glucocorticoids metabolites also pose the question on “why households in group one are more aroused environments than group two households?” Although they were equally multi-cat houses, it is important to note that *spraying* households had significantly more cats than *toileting* ones (Two samples t-test, $p=0.011$). Furthermore, outside access (previously reported to be associated with problem behaviours such as aggression – RAMOS; MILLS, 2009; LEVINE et al., 2005) was available in 5 *spraying* and none of the *toileting* houses. Higher arousal could at least in parts be explained by these factors or by something else not investigated in this research (e.g. owner attitudes towards the cats, cat group composition: time living together, kinship, time when last cat was incorporated etc.).

Although additional experiments are required to verify the effects of *spraying* on already aroused cats, the present result supports the recommendation that simply restricting the *spraying* behaviour (e.g. by punishment) should not be part of the treatment plan for “sprayer” cats as this will not reduce arousal.

Unfortunately, more information about emotional states of participant cats are lacking due to the unreliability of our method of evaluation.

5.5 Behavioural style: cause, consequence or what?

Despite the richness, integrative nature and foundation on intimate knowledge of the animal (MEAGHER, 2009), opinion of the owners about their cats' behavioural style (i.e. personality), at least in the present research, did not produce sufficiently reliable data. Such a result is in contrast to previous studies which examined the reliability dog and cat owner scoring in relation to other features of their animals (GERMAN et al., 2006; WOJCIECHOWSKA et al., 2005). The former involved owners' judgment on the body condition of the animals, while the latter was about quality of life; only a moderate reliability was found in the latter, thus showing that when it comes to behaviour (versus physical features) opinions may be more prone to systematic biases.

Our finding, however, should not discourage the use of owners' ratings; but rather they emphasize the need for more research on this matter; we believe there could have been a flaw in the questionnaire design. By including similar questions about "cats in general" along all the seven domains asked about the participant cats, (in an attempt to produce more standardized scores) we may have confused owners. Nonetheless, the last three questions on composite personalities ("bossy", "timid, "easy going") were not accompanied by questions on "cats in general" and owners still did not show reliability when their scores were compared with the ones from another family member. In this case, however, not all households had a second family member to be interviewed and so comparisons were undertaken with small numbers. Clearly, more investigations are deserved and more efforts should be put into designing more reliable scales before giving up on the owners' perception about their cats' personality.

The present result restricted our investigation about a possible link between inappropriate urination and personality. For a comparison between "toileters" and

“sprayers” to be made, only their scores for both *hostile to people* and *tenseness* could be taken into consideration, as a strong correlation was found for both groups regarding reliability of answers on these two domains. Cats from *spraying* households did not significantly differ to cats from *toileting* households (Two samples t-test, $p=0.358$, $p=0.410$, respectively for *hostile to people* and *tense*). As it stands, it seems reasonable to conclude that neither of the problems seem to rely on these aspects of personality. Indeed, inappropriate *toileting* was not expected to be linked to a behavioural style, but we expected some behavioural distinction in the “sprayers” (WESTROPP; BUFFINGTON, 2004; BOWEN; HEATH, 2005; CALIXTO; JUSTEN, 2007). Having selected the strategy of *spraying* one may suggest “sprayers” were more proactive cats; this does not necessarily translate into any particular tendency concerning hostility to people and tenseness, though.

Personality of the participant cats could alternatively be evaluated through the videos with the limitation of being a single situation only; cats might show variation across situations (KIDDIE; CASEY, 2009) and thus careful interpretation should be made when talking about “personalities” seen on the videos. Overall, no differences between “cases” and “controls” regarding their behaviour in a standard test were seen in any of the groups. However, an indication of a more frequent *meowing* among the “sprayers” and a more frequent *moving frightened* among “toileters” deserves further investigation. When all cats in each group are considered together, and an in-between subjects comparison was made, two differences arisen:

1 - When looking at *move* \ (*move* + *stop*) proportions, cats from *spraying* houses spent significantly more time moving (in proportion to stop) (Kruskall-Wallis test, $p=0.011$). Cats from *spraying* houses moved about relatively more with less stationary elements in the case of “sprayers”;

2 -When looking at time spent *stopped* (duration), cats from *toileting* houses spent longer time than cats from *spraying* houses (Kruskall-Wallis test, $p=0.050$).

A more active type of behaviour was manifested by cats from “spraying” households overall, this could be a result of their higher arousal states. A larger sample size is needed for a better understanding on whether “sprayers” differ in this

regard. Their common arousal states may have masked a more clear indication of an impulsive like trait among “sprayers”.

5.6 Social behaviour: does it matter?

Perception of the owners of the *dominance* and *submissiveness* of their cats within the multi-cat group were not tested for reliability as done with the personality questionnaire. However, besides the owners rating, the two concepts were indirectly investigated throughout the social matrices which were also answered by the same owners. Considering that with both instruments (i.e. direct scores and social matrices), and for the two groups, “case” cats did not appear to be any different from their “controls”, we can suggest that owners scores for *dominance* and *submissiveness* were relatively valid. In other words, data extracted from the social matrices led to the same conclusions taken from the owners’ scores.

As to the social matrices which were evaluated via Dominance and Affiliative Trees (IZAR, 1997), “cases” and “controls”, from both groups, did not differ in terms of frequency of affiliative interactions, nor in relation to agonism; levels of dominance based on a probable linear dominance hierarchy also did not differ between “cases” and “controls” in either of the groups. But did the households in the two groups differ in terms of occurrence of agonistic and affiliative relationships? *Spraying* and *toileting* households had similar occurrence of affiliation and agonism among the habitant cats (Two samples t-test t-test, $p=0.077$; Kruskal-Wallis, $p=0.356$) respectively for frequency of affiliation and frequency of agonism. It has to be considered that in group 1 (*spraying*) there was a positive correlation between number of cats and frequency of affiliative relationships (Linear Regression $p=0.020$); the same was not found for group 2 (*toileting*) (Linear Regression, $p=0.649$). This explains why we almost found a significant difference between *spraying* and *toileting* houses with regard to affiliation: the former households had significantly more cats.

The previously proposed argument that cats from *spraying* houses could be more aroused due to a higher number of cats would be justified by a higher occurrence of conflicts in the *spraying* houses. This was not identified, though. But one may still think that a higher frequency of encounters between the cats, for instance, would justify the *spraying* in households from group 1. Or, a conflicted relationship between the human family and the problem cat may underlie *spraying* in multi-cat houses. Further studies in this direction are therefore required.

Clearly, in the present research, it was demonstrated that neither urine *spraying* nor inappropriate *toileting* is obviously related to inter-cat social relationship. At least in multi-cat households inappropriate urination does not appear to be a dominance or submissiveness manifestation.

5.7 Final Conclusions and Summary

- Ruling out medical problems is equally relevant in cases of urine *spraying* and inappropriate *toileting*;
- Alterations of the upper urinary system may be linked to inappropriate urination manifested as either *spraying* or inappropriate *toileting*;
- Households with cats exhibiting urine *spraying* seem to be highly aroused environments. Appropriate management applied to those households should be focused on alleviating stress for all the cats;
- *Toileting* and *Spraying* do not seem to be linked to social behaviour or behavioural style. But *spraying* may arise in households with more cats, where more affiliation seem to exist, and may be seen as a coping strategy;
- *Future research should focus on a possible link between upper urinary tract alterations and inappropriate urination; if this in fact exists, an even more careful medical check-up will be required in cases of inappropriate urination. Furthermore, environmental management and feline care should be better investigated in multi-cat households as they seem to be on the root of both medical and behavioural problems.*

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Appendices

APPENDIX A. Leaflet which served research advertisement.

Seu gato se recusa a usar a caixa sanitária?

Apesar de conhecidos pela limpeza, a micção e defecação fora da caixa sanitária são problemas comuns entre os gatos. Longe de vilões e revanchistas, muitos gatos que se recusam a usar o banheiro estão na verdade sofrendo de estresse ou apresentam doença urinária. Podemos te ajudar a ajudar o seu gato!

Somos veterinários da Faculdade de Medicina Veterinária da Universidade de São Paulo interessados no estudo do comportamento de gatos que vivem em grupos e urinam fora da caixa sanitária. Precisamos da sua ajuda!

Se você reside em São Paulo, possui de 3 a 9 gatos em casa e um (ou mais) deles apresenta micção fora da caixa sanitária, entre em contato conosco. A participação em nossa pesquisa é gratuita e lhe forneceremos informação e total apoio na resolução deste problema.

Dra. Daniela Ramos
daniela.ramos@usp.br

Apoio: Dr. Alexandre G. T. Daniel

Supervisão: Prof. Archivaldo Reche Junior

APPENDIX B. About the households, the toilets and the urination problem.

GROUP 1	URINE SPRAYING <i>(11 "case-control" dyads – 9 households)</i>	
The "households"	<i>Type of environment</i>	All houses
	<i>Number of people</i>	From 1-5 people (average: 2.8 people per household)
	<i>Presence of children</i>	In only 3 households (1-2 children/house)
	<i>Presence of dogs</i>	In 6 households (1dog/house)
	<i>Outside access</i>	In 5 households; in other 3 households there was an enclosed yard
The "problem"	<i>How long problem had been present?</i>	Most of them for 2 years at least – in two cases about 1 year only
	<i>Progression</i>	In 5 cases "worsening", in 5 cases "no changes" and in 1 case "improving"
	<i>Owners' suspicious</i>	Different opinions: fear, dirtiness, revenge; two owners mentioned territorialism, increase number of cats in the house, conflicts
	<i>Owners' attitude</i>	Most used reprimands and different sorts of chemicals to clean the soiled areas; one owner declare hitting the cat and one mentioned the use of synthetic pheromone (with no success)
	<i>Frequency of occurrence</i>	In 6 cases "daily", in 4 cases "2-3 times/week" and in 1 case "monthly"
	<i>Do other cats soil the house?</i>	"Yes" in 6 households
The "toilet"	<i>Number of "litter boxes"</i>	4 on average
	<i>Number of "Litter boxes" location</i>	2 on average
	<i>Type of substrate</i>	Different types: shaves of wood (1), sand (1), newspaper (2), manioc flower (1), commercial litter (4 – being scented in only one household)
	<i>Excretes removal</i>	6 "at least 3 times a day", 3 "once a day"
	<i>Complete cleaning</i>	4 "once a week", 3 "once a day", 1 "every other day", 1 "never"

GROUP 2	INAPPROPRIATE TOILETING BEHAVIOUR <i>(12 “case-control” dyads – 11 households)</i>	
The “households”	<i>Type of environment</i>	6 houses, 5 apartments
	<i>Number of people</i>	From 1-4 people (average: 2.5 people per household)
	<i>Presence of children</i>	In none of the households
	<i>Presence of dogs</i>	In 6 households (2.8 dog/house)
	<i>Outside access</i>	In 5 households there was an enclosed yard and in 4 a balcony; in none there was free outside access
The “problem”	<i>How long problem had been present?</i>	Most of them for 2 years at least – in two cases about 1 year only
	<i>Progression</i>	In 2 cases “worsening”, in 9 cases “no changes” and in 1 case “improving”
	<i>Owners’ suspicious</i>	Different opinions: fear, revenge, dominance, attention seeking, weirdness, over attachment, “litter box” dislike; one of the owners mentioned the “litter box” was too dirty for the cat to use
	<i>Owners’ attitude</i>	Most used reprimands and different sorts of chemicals to clean the soiled areas; one owner hit the cat and three increased the number of box; one declared using positive reinforcement when cat used the “litter box”
	<i>Frequency of occurrence</i>	In 5 cases “daily”, in 4 cases “2-3 times/week” and in 3 cases “once a week”
	<i>Do other cats soil the house?</i>	“Yes” in 4 households
The “toilet”	<i>Number of “litter boxes”</i>	3 on average
	<i>Number of “Litter boxes” location</i>	1.9 on average
	<i>Type of substrate</i>	11 commercial litter (scented in two households)
	<i>Excretes removal</i>	8 “1-2 times a day”, 2 “several times a day”, 1 “every other day”
	<i>Complete cleaning</i>	4 “once a week”, 4 “every three days”, 2 “every other day”, 1 “once a month”

APPENDIX C. Questions filled in by the research during the visits and which lead to the behavioural diagnosis.

DIAGNÓSTICO COMPORTAMENTAL

1. USA A CAIXA DE ELIMINAÇÃO? PARA O QUE?	
2. URINA E FEZES FORA DA CAIXA?	
3. FREQUÊNCIA DE ELIMINAÇÃO INAPROPRIADA (diariamente, Semanalmente, Mensalmente)	
4. QUANDO A ELIMINAÇÃO NORMALMENTE ACONTECE?	
5. LOCALIZAÇÃO DA ELIMINAÇÃO INAPROPRIADA NA CASA? LOCALIZAÇÃO ESPECÍFICA DA ELIMINAÇÃO INAPROPRIADA NO RECINTO?	
6. TIPO DE SUPERFÍCIE ONDE A ELIMINAÇÃO INAPROPRIADA É DEPOSITADA (MATERIAL) (VERTICAL/HORIZONTAL)?	
7. TIPO DE POSTURA DE ELIMINAÇÃO INAPROPRIADA?	
8. TAMANHO DA ELIMINAÇÃO INAPROPRIADA (MANCHAS, POÇAS)?	
9. COMPORTAMENTO DO GATO NA CAIXA DE AREIA?	
10. RELACIONAMENTO COM OUTROS MEMBROS DA FAMÍLIA INCLUINDO PETS? (ESTRESSE?)	

Diagnóstico: () SPRAYING () TOILETING

APPENDIX D. Feline temperament questionnaire filled out by the owners.

Teste de avaliação de temperamento para gatos domésticos

Este questionário tem a finalidade de auxiliar na avaliação do temperamento do seu gato. Para isso, responda aos itens abaixo de acordo com a sua observação sobre o comportamento dele, identificando, na linha preta, onde você acha que ele melhor se posiciona. Em vermelho, aponte onde estariam os gatos de um modo geral. Tome como base apenas a sua percepção, não consultando a opinião de outros membros da família.

a) ATIVO – se movimenta com bastante freqüência

Two horizontal scales are provided for rating the frequency of use of 'NADA' and 'TOTALMENTE'. Each scale consists of a horizontal line with 11 vertical tick marks, creating 10 equal intervals. The left end of each scale is labeled 'NADA' and the right end is labeled 'TOTALMENTE'. The top scale is in black text, and the bottom scale is in red text.

b) CURIOSO – se aproxima e explora qualquer mudança no seu ambiente

c) SOCIÁVEL COM PESSOAS – inicia aproximação e/ou contato com pessoas

NADA TOTALMENTE

NADA TOTALMENTE

d) MEDROSO COM PESSOAS – se afasta prontamente de pessoas

NADA TOTALMENTE

NADA TOTALMENTE

e) HOSTIL COM PESSOAS – reage à aproximação de pessoas com ameaça ou efetivamente machuca quem se aproxima

NADA TOTALMENTE

NADA TOTALMENTE

f) TENSO - demonstra postura e movimento retraído

NADA TOTALMENTE

NADA TOTALMENTE

**g) EQUILIBRADO COM GATOS – reage a outros gatos com calma, sendo
difícilmente perturbado**

NADA TOTALMENTE

NADA TOTALMENTE

h) Quanto você acha que seu gato é MANDÃO?

NADA TOTALMENTE

i) Quanto você acha que seu gato é TÍMIDO?

NADA TOTALMENTE

j) Quanto você acha que seu gato é TRANQUILO?

NADA TOTALMENTE

APPENDIX E. Feline social matrices filled by the researcher in conjunction with the owners during visits to the households.

Teste de avaliação de status social para gatos domésticos

COMPORTAMENTOS AGRESSIVOS CONTRA O OUTRO: ameaça (rosna, assopra) ou literalmente ataca (morde, arranha) o outro gato.

esq - dir	1	2	3	4	5	6	7	8	9
1									
2									
3									
4									
5									
6									
7									
8									
9									

FREQUÊNCIA DE OCORRÊNCIA: quase nunca (N), raramente (R), ocasionalmente (O), frequentemente (F), quase sempre (S)

ESFREGAR-SE NO OUTRO: esfrega parte (ou partes) do seu corpo no outro gato.

esq-dir	1	2	3	4	5	6	7	8	9
1									
2									
3									
4									
5									
6									
7									
8									
9									

FREQUÊNCIA DE OCORRÊNCIA: quase nunca (N), raramente (R), ocasionalmente (O), frequentemente (F), quase sempre (S)

DORMIR JUNTO COM O OUTRO: dormelado a lado com o outro gato,

esq-dir	1	2	3	4	5	6	7	8	9
1									
2									
3									
4									
5									
6									
7									
8									
9									

FREQUÊNCIA DE OCORRÊNCIA: quase nunca (N), raramente (R), ocasionalmente (O), frequentemente (F), quase sempre (S)

LAMBERO OUTRO: lambe parte (ou partes) do corpo do outro gato,

esq-dir	1	2	3	4	5	6	7	8	9
1									
2									
3									
4									
5									
6									
7									
8									
9									

FREQUÊNCIA DE OCORRÊNCIA: quase nunca (N), raramente (R), ocasionalmente (O), frequentemente (F), quase sempre (S)

APPENDIX F. Feline social status questionnaire filled out by the owners.

GATO 1 – CASO

Gostaríamos de saber a sua opinião em relação ao status social do seu gato dentro do grupo. Para isso, responda aos itens abaixo de acordo com a sua observação sobre o comportamento dele dentro do grupo, identificando, na linha, onde você acha que ele melhor se posiciona. Tome como base apenas a sua percepção, não consultando a opinião de outros membros da família.

a) Quanto você acha que seu gato é DOMINANTE?

NADA TOTALMENTE

a') É tão DOMINANTE que chega a aterrorizar outros membros do grupo, já que usa de MUITA agressividade para com eles. () Sim () Não

b) Quanto você acha que seu gato é SUBMISSO?

NADA TOTALMENTE

b') É tão SUBMISSO que chega a ser aterrorizado por outros membros do grupo; frequentemente é vítima de ataques e, inclusive, mostra-se excluído do grupo. () Sim () Não

GATO 2 – CONTROLE

Gostaríamos de saber a sua opinião em relação ao status social do seu gato dentro do grupo. Para isso, responda aos itens abaixo de acordo com a sua observação sobre o comportamento dele dentro do grupo, identificando, na linha, onde você acha que ele melhor se posiciona. Tome como base apenas a sua percepção, não consultando a opinião de outros membros da família.

c) Quanto você acha que seu gato é DOMINANTE?

NADA TOTALMENTE

a') É tão DOMINANTE que chega a aterrorizar outros membros do grupo, já que usa de MUITA agressividade para com eles. () Sim () Não

d) Quanto você acha que seu gato é SUBMISSO?

NADA TOTALMENTE

b') É tão SUBMISSO que chega a ser aterrorizado por outros membros do grupo; frequentemente é vítima de ataques e, inclusive, mostra-se excluído do grupo. () Sim () Não

APPENDIX G. Graphic representation of social relationships followed by
 Dominance Tree for Household 1 (Group 1 – “sprayers”: speedy-cadu dyad)

APPENDIX H. Graphic representation of social relationships followed by Dominance Tree (bottom left) and Affiliative Tree (bottom right) for Household 2 (Group 1 – “sprayers”: gorda-quick and med-foguinho dyads)

APPENDIX I. Graphic representation of social relationships followed by Dominance Tree (bottom left) and Affiliative Tree (bottom right) for Household 3 (Group 1 – “sprayers”: marcel-luci dyad)

APPENDIX J. Graphic representation of social relationships followed by Dominance Tree (bottom left) and Affiliative Tree (bottom right) for Household 4 (Group 1 – “sprayers”: oscarito-lilea dyad)

APPENDIX K. Graphic representation of social relationships followed by Dominance Tree and Affiliative Tree for Household 5 (Group 1 – “sprayers”: mussum-mi dyad)

APPENDIX L. Graphic representation of social relationships followed by Dominance Tree (bottom left) and Affiliative Tree (bottom right) for Household 6 (Group 1 – “sprayers”: guto-thiago dyad)

APPENDIX M. Graphic representation of social relationships followed by Affiliative Tree for Household 7 (Group 1 – “sprayers”: nino-pitulo dyad)

APPENDIX N. Graphic representation of social relationships followed by Dominance Tree (bottom left) and Affiliative Tree (bottom right) for Household 8 (Group 1 – “sprayers”: fofa-cotoca and ronin-macaca dyads)

APPENDIX O. Graphic representation of social relationships followed by Dominance Tree (bottom left) and Affiliative Tree (bottom right) for Household 9 (Group 1 – “sprayers”: mitchu-et dyad)

APPENDIX P. Graphic representation of social relationships followed by Dominance Tree (bottom left) and Affiliative Tree (bottom right) for Household 1 (Group 2 – “toileters”: monalisa-bia dyad)

APPENDIX Q. Graphic representation of social relationships followed by Affiliative Tree for Household 2 (Group 2 – “toileters”: tonico-francesco dyad)

APPENDIX R. Graphic representation of social relationships followed by Dominance Tree (bottom left) and Affiliative Tree (bottom right) for Household 3 (Group 2 – “toileters”: mila-horacio dyad)

APPENDIX S. Graphic representation of social relationships followed by Dominance Tree (bottom left) and Affiliative Tree (bottom right) for Household 4 (Group 2 – “toileters”: natasha-floripa dyad)

APPENDIX T. Graphic representation of social relationships followed by Dominance Tree (bottom left) and Affiliative Tree (bottom right) for Household 5 (Group 2 – “toileters”: agata-mel dyad)

APPENDIX U. Graphic representation of social relationships followed by Dominance Tree (bottom left) and Affiliative Tree (bottom right) for Household 6 (Group 2 – “toileters”: darah-thor and stephania-pollux dyads)

APPENDIX V. Graphic representation of social relationships followed by Dominance Tree (bottom left) and Affiliative Tree (bottom right) for Household 7 (Group 2 – “toileters”: brisa-toquinho dyad)

APPENDIX W. Graphic representation of social relationships followed by Dominance Tree and Affiliative Tree for Household 8 (Group 2 – “toileters”: mel-babalu dyad)

APPENDIX X. Graphic representation of social relationships followed by Dominance Tree and Affiliative Tree for Household 9 (Group 2 – “toileters”: marie-chiara dyad)

APPENDIX Y. Graphic representation of social relationships followed by Dominance Tree and Affiliative Tree for Household 10 (Group 2 – “toileters”: luã-mila dyad)

APPENDIX Z. Graphic representation of social relationships followed by
Dominance Tree for Household 11 (Group 2 – “toileters”: diná-cléo dyad)