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Factors influencing stress and fear-related behaviour of cats during veterinary examinations

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ABSTRACT

This study documented behaviour of cats during veterinary examinations investigating possible associations between their behaviour and age, gender, temperament, clinical parameters and familiarity with pet carriers, car rides and veterinary clinics. Background information (i.e. temperament and familiarity with pet carriers, car rides and veterinary clinics), signalment, clinical parameters and behaviour were recorded for 95 cats during a veterinary examination. Their behaviour was analysed and a behavioural profile was attributed (alert, anxious, frightened, irritated, agonistic). Aggressive cats tended to exhibit more scratching/attempt to scratch and hard stare behaviour. Half of the cats vocalised and cats vocalised more when they were not familiar with pet carriers ($p = .010$) and car rides ($p = .005$). Heart rate was higher than normal and was positively associated with mydriasis ($p = .018$) shown by the 85% of the cats. Males vocalised more than females ($p = .007$) who also had a lower total behavioural score ($p = .002$). Age was associated with the behaviour profile: young cats fell in the anxious profile, and were more likely to try to escape, and elderly cats exhibited a frightened and irritated profile and were more likely to show scratching, tail lashing and piloerection (all $p < .05$). Whilst cats first experiencing a veterinary examination attempted to escape more, those that were not familiar with car rides had higher behavioural scores ($p = .05$). Overall, veterinary examination was confirmed to be a stressful situation for cats in particular for those not familiar with pet carriers and car rides.

HIGHLIGHTS

- Cats' physiological and behavioural responses during veterinary examinations were documented.
- Young cats were more anxious, while elderly cats were irritated and showed more scratching, tail lashing and piloerection.
- Cats not familiar with car rides and veterinary clinics showed more vocalisation, hissing, ears flat back and escape attempts.

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Cat; behaviour; fear; veterinarian; transport; training

Introduction

Cats become highly stressed during visits to the veterinary clinic. Italian cat owners reported that their cats were stressed based on physiological parameters, such as an increase of heart rate and presence of dilated pupils, and behavioural changes. In particular, cats were retained to be under stress when hyper-vocalising, ears back, inappropriate or increased urination and defaecation, escape attempts and aggressive behaviour were shown. Cat owners also reported that their cats were particularly stressed while travelling or

during veterinary examinations (Mariti et al. 2017). For this reason, their owners reported that they prefer not to consult a veterinarian at a clinic or hospital to avoid this stress (Volk et al. 2011). Veterinary examinations are challenging also for staff at risk of being scratched or bitten by agitated and aggressive cats (Jeyaretnam et al. 2000; Rodan et al. 2011; Hammerle et al. 2015). Many cat owners have also reported that their animals have shown signs of impaired welfare during all stages of a veterinary visit and hostility for several days after they return home, which is particularly undesirable for sick or recovering animals (Mariti et al.

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 Supplemental data for this article can be accessed [here](#).

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2016). Even a single visit to the veterinary clinic can have a dramatic impact on a cat's long-term behaviour (Volk et al. 2011). The stress caused by the veterinary environment is therefore undesirable not only for the effects on behaviour but also for the adverse effects on the immune system, speed of recovery of the animal and for the risk of injury to veterinary staff.

In the literature, there are a variety of suggestions to minimise stress of cats during veterinary examinations. Short-acting drugs to sedate the animal have been proposed (Landsberg et al. 2013). However, adverse reactions to these drugs are factors limiting their use. Cats, in comparison to other species, are indeed deficient in some metabolic pathways used to metabolise psychoactive and analgesic drugs. This deficiency can lead to toxicity or partial or total lack of effect (van Haaften et al. 2017). An appropriate use of medications and anxiolytics before veterinary examination may however be a substantial help in minimising stress in patients (Lloyd 2017). Recently, Fear Free[®] certificated clinics (<https://fearfreepets.com/>) published a guide to use choosing the best pre-visit medications which can be used to sedate or reduce anxiety in animals that must undergo veterinary examination. Among the suggested medications, gabapentin showed to be safe and effective for treating cats to help reduce stress and aggression prior and during veterinary examination (van Haaften et al. 2017). Although often viewed with suspicion, other complementary therapies, such as Bach flowers (tiny amounts of flower essence used to calm emotional animals), Reiki and Shen (the therapist's channelling of 'energy' into the patient) have been implemented in some veterinary practices to treat a range of animal anxiety problems, including stress and fear induced by veterinary examinations in cats (Crowell-Davies & Landsberg 2012). However, recently, understanding of cat behaviour and physiology has improved, leading to superior clinical management of this species, and behavioural methods have also been used as rational treatment protocols to manage cats during veterinary examinations (Bowen and Heath 2005; Yin 2009; Overall 2013). Educating guardians and veterinarians to minimise stress during every stage of a visit has been also recommended (Mariti et al. 2016).

Prevention seems also to be useful to minimise stress and fear in cats. Preparing the patient before its visit has been suggested for preventing anxiety build-up and relieve stress on arrival at the hospital (Yin 2009). Dogs and cats quickly learn to associate frightening or painful experiences with veterinary hospital and staff through classic conditioning. It has been

reported that many cats hid in the presence of the pet carrier and vocalised during the journey to the veterinary hospital (Volk et al. 2011). However, classical conditioning and classic counter-conditioning could reduce the frequency of those unwanted behaviours (Overall 2013). This approach could also be useful for patients who present themselves as emergencies, as training could be done preventively, reducing stress during a subsequent emergency visit. The American Association of Feline Practitioners (AAFP) and the International Society of Feline Medicine (ISFM) proposed feline-friendly handling guidelines for the management of cats. Those guidelines illustrate how to prepare clients and cats for travel to the hospital and the progressive signs of fear and anxiety in cats from early to late stages (Rodan et al. 2011). A proper carrier training has been proven to reduce stress on transport to a veterinary practice (Pratsch et al. 2018). The hypothesis of this study was therefore that familiarity with pet carriers, car rides and veterinary clinics would minimise stress and fear-related behaviour in cats subjected to veterinary examination, even if intrinsic factors, such as age, gender and temperament would also affect cat behaviour. The aim of this study was to document stress and fear-related behaviour of cats, with different background, during veterinary examinations and to investigate possible associations between their behaviour and age, gender, temperament, physiological parameters and familiarity with pet carriers, car rides and veterinary visits.

Materials and methods

This prospective cohort study was carried out with the approval of the animal ethics committee of the Department of veterinary medicine, the University of Bari, Italy (CESA n. 11/2020).

Experimental protocol

Data collection was performed on every cat taken to visit a private conventional veterinary clinic without a fear-free certification, in the South of Italy for three months. A total of 95 cats with scheduled appointments were examined. The cats were transported to the veterinary clinic in a pet carrier and were let in the inspection room with the owner as soon as they arrived. The cats and cat's owners did not stay in the waiting room in order to minimise distress or contact with other animals. After having obtained the cat owner's consent to be part of the study, a single observer (A.C.) with experience in feline behaviour,

Table 1. Ethogram used during the study. The behaviour was recorded using a 1/0 sampling method.

Behaviour	Definition
Mydriasis	Cat showed very dilated pupils (Nibblett et al. 2015)
Slit pupils	Cat showed slightly narrowed or oblong pupils (Nibblett et al. 2015)
Alert ears	Cat showed ears upright and oriented forward (Nibblett et al. 2015)
Ears pressed flat back	Cat showed ears pressed flat back against the head (Nibblett et al. 2015)
Escape attempt	Attempt by the cat to struggle while restrained, or move purposefully and rapidly away from the handler at any time (Nibblett et al. 2015)
Tail lashing	A rapid flick of the tail in either a side to side or up to down motion (Cameron-Beaumont 1997)
Scratching/Attempt to scratch	The cat scratched the veterinarian/handler or the cat lifted his paw rapidly trying to scratch without making any physical contact
Crouching in carrier	Crouching close to the floor of the container
Hard stare	Cat gazed fixedly at (something) and is not easily distracted (Stanton et al. 2015)
Urination	Cat released urine on the ground/table while in a squatting position
Defaecation	Cat released faeces on the ground while in a squatting position (Stanton et al. 2015)
Biting/Attempt to biting	Cat bites or attempts to bite (Stanton et al. 2015)
Vocalising	Cat produced sounds or calls, originating from the throat and mouth; meow, meow loudly, yowl or growl (Nibblett et al. 2015)
Hiss	The cat opened its mouth, forcing the burst of air out with a hissing sound (Nibblett et al. 2015)
Piloerection	Cat raises the hairs on the nape of its neck, shoulder, back or tail, so that the fur is standing erect (Stanton et al. 2015)

collected the data at the triage. The observer used an assessment form including the following parameters: name, age, breed and gender of the cat and the reason for the visit. The observer also asked every owner to fill in a multiple-choice questionnaire, containing the following questions: temperament of the subject (Aggressive/Non-aggressive), first visit (Yes/No), familiarity with the pet carrier (Yes/No), familiarity with car rides (Yes/No).

During the veterinary examination, physiological parameters were measured by a veterinarian and noted down by the observer. Briefly, heart rate (HR) was measured for a 15-second interval using a stethoscope (Littman classic III, China), and multiplied by four to obtain HR measured in beats per minute (bpm). Respiratory rate (RR) was measured as breaths per minute (bpm) by observing the number of flank movements in a 1-minute interval. Rectal temperature (RT) was taken with a lubricated digital thermometer (Henry Schein, USA). In order to standardise as much as possible the examinations, HR, RR and RT have always been measured following this order.

The same observer, present throughout the visit, recorded the behavioural patterns. These were recorded using the ethogram shown in Table 1. Those selected stress and fear-related behaviours were recorded with a 1/0 (present/absent) ethogram throughout the veterinary examination.

Based on the behavioural observation and the presence of some key behaviours, the same operator at the end of the visit gave to each of the observed cat one profile (Table 2) modified from the Arizona Humane Society (2017).

Statistical analysis

The collected data were divided into explanatory variables (Table 3). Age was split into three categories: (i) young; less than 10 months, (ii) adult; from 11 months to 8 years, (iii) elderly; more than 9 years. Breed was split into two categories: European and non-European (Persian, Burmese, Norwegian Forest, Siamese and Somali, Carthusian). The reasons for the veterinary visits were grouped into the following categories: general visit, visit due to a specific clinical sign (related to the respiratory, gastrointestinal, or urinary system), visit for a veterinary diagnostic procedure (X-ray, ultrasound), visit for a veterinary invasive procedure (vaccination/blood sample), or other (i.e. lameness, euthanasia, fluid-therapy, and alopecia). The responses to the cat owner survey and the physiological parameters were left as initially registered. Descriptive statistics of the data were calculated using Statulor online tool (KKhatkar and Dhand 2014).

Before proceeding with further analysis, as some behaviours were mutually exclusive, the data set was consolidated. In particular, when a cat showed both alert ears (1) and ears flat back (1), 1 was kept only for ears flat back, and when the cat showed the presence of both mydriasis and slit pupils, 1 was kept only for slit pupils. Scratching and attempt to scratch were also combined. After those changes, a model was derived for each cat behaviour; the outcome was binary (1/0, presence/absence). The explanatory variables explored included age, gender, breed, HR, RR, RT, reason, temperament, first visit, carrier, and travelling. Univariate logistic regression was performed using GenStat[®] Version 14 (VSN International, Hemel

Table 2. Type of profile attributed to each cat based on the behaviour showed during the veterinary examination of 95 cats taken to a veterinary clinic in the south of Italy.

Type of profile	Description
Alert	During the visit, the cat was mainly alert, with ears erect and upright most of the time, but overall relaxed.
Anxious	During the visit, the cat had its ears sideways or back and pupils very dilated most of the time. It was crouching close to the floor or the container, showing some tail lashing. It tried to escape.
Frightened	During the visit, the cat had its ears pressed flat and pupils very dilated most of the time; it was crouching close to the floor or the container. It tried to escape. It vocalised (i.e. yowl, growl) and hissed showing some piloerection.
Irritated	During the visit, the cat had its ears turned back and pupils very dilated, often showing tail lashing. It vocalised (i.e. yowl, growl) and hissed, and threatened to scratch.
Agonistic	During the visit, the cat had its ears pressed flat back and slit pupils. Its tail may be up or down or lashed side to side. It showed piloerection, hard stare, yowl, spit, scratching or attempt to scratch, bite or attempts to bite

Table 3. Classification of the explanatory variables considered in the study with data collected from 95 cats taken to a veterinary clinic in the south of Italy.

Name	Description	Values
Age	Age of the cats	Young, adult, elderly
Gender	Gender of the cat	Male, castrated male, female, neutered female
Breed	Cat breed	European, non-European
Reason	Reason why the owner brought the cat to the veterinary clinics	General visit, surgery, gastro-enteric problem, respiratory problem, urinary problem, vaccine/blood sample, diagnostic (X-ray or ultrasound), other (lameness, alopecia, euthanasia, parental fluids)
Temperament	The temperament of the cat reported by the owner	Aggressive, non-aggressive
First visit	If the veterinary visit was the first-ever done	Yes, No
Carrier	If the cat was familiar with the pet carrier	Yes, No
Travelling	If the cat was familiar with car rides	Yes, No

Hempstead, UK), *P* values were calculated using Wald Test and data are expressed as Odds Ratio (OR), 95%CI and Wald-test *p*.

Using the consolidated dataset, the behavioural parameters were also summed to generate a 'total behavioural score'. In order to identify possible associations, a univariate multifactorial logistic analysis was therefore conducted by using the 'total behavioural score' as the dependent variable and the age, gender, reason, temperament, first visit, carrier, and travelling as fixed factors. Initially, the model also included the cat breed as a fixed effect. However, since no significant effect of breed was found, the breed was no longer considered among the independent variables. The univariate multifactorial logistic analysis was performed in the R environment (R Core Team 2020), with the glm function of the stats package. Data are expressed as (OR) and the 95% confidence interval (95% CI) estimated using basic functions in the stats package.

Moreover, to identify possible associations between the cat profile and the explanatory variables, a Chi-square test was performed using GenStat[®] Version 14 (VSN International, Hemel Hempstead, UK).

Finally, to detect and represent underlying structures in the data set, a multiple correspondence analysis (MCA) was also performed. MCA is an unsupervised type analysis that identifies patterns in a data set by combining the original variables into new descriptive components (named dimensions). Each dimension is determined by a specific combination of the original variables, which, based on their weight in each dimension, contribute in explaining the total variance that is noticed in the sample. The MCA was performed considering the cat profile, all the binary variables describing the behaviours, the cat's age, gender, reason for the visit, character, familiarity with the pet carriers and car rides, and whether it was or not the first veterinary visit. The MCA was performed using the factoextra package, and the results were plotted using the packages gplots and grDevices in the R environment. The MCA results were then used to perform a Hierarchical Clustering on Principal Components (HCPC) analysis using HCPC function of FactoMineR package in the R environment. The latter analysis allows identifying (i) a hierarchy in the importance of the variables describing the sample variability, (ii) different clusters in the observations and associate

Table 4. Descriptive statistics of the data collected from 95 cats taken to a veterinary clinic in the south of Italy.

Variable Name	Category	Count	Percent
Age	adult	43	45.4
	elderly	26	27.3
	young	26	27.3
	Total	95	100
Gender	male	27	28.4
	neutered	27	28.4
	female	25	26.3
	castrated	16	16.9
	Total	95	100
Breed	European	82	86.3
	Non-European	13	13.7
	Total	95	100
Reason	general visit	25	26.3
	vaccine/blood	16	16.8
	other	14	14.7
	gastroenteric	13	13.7
	surgery	8	8.4
	x-ray/ultrasound	8	8.5
	respiratory	6	6.3
	urinary	5	5.3
	Total	95	100
	Temperament	aggressive	10
non-aggressive		85	89.5
First visit	Total	95	100
	no	72	76.6
	yes	22	23.4
Carrier	Total	94	100
	Missing Values	1	1.0
	no	47	50.5
	yes	46	49.5
Travelling	Total	93	100
	Missing Values	2	2.1
	no	55	59.8
	yes	37	40.2
Mydriasis	Total	92	100
	Missing Values	3	3.2
	0	14	14.7
	1	81	85.3
Slit pupils	Total	95	100
	0	78	82.1
	1	17	17.9
Alert ears	Total	95	100
	0	14	14.7
	1	81	85.3
Ears pressed flat back	Total	95	100
	0	77	81.1
	1	18	18.9
Escape attempt	Total	95	100
	0	64	67.4
	1	31	32.6
Tail lashing	Total	95	100
	0	79	83.2
	1	16	16.8
Scratching/Attempt to scratch	Total	95	100
	0	72	75.8
	1	23	24.2
Crouching in carrier	Total	95	100
	0	84	88.4
	1	11	11.6
Hard stare	Total	95	100
	0	90	94.7
	1	5	5.3
Biting/Attempt to bite	Total	95	100
	0	86	90.5
	1	9	9.5
Vocalising	Total	95	100
	0	48	50.5
	1	47	49.5
Hiss	Total	95	100
	0	70	73.7

(continued)

Table 4. Continued.

Variable Name	Category	Count	Percent
Piloerection	1	25	26.3
	Total	95	100
	0	70	73.7
Profile	1	25	26.3
	Total	95	100
	agonistic	29	30.5
Profile	frightened	25	26.3
	anxious	16	16.9
	irritated	14	14.7
	alert	11	11.6
	Total	95	100

them with the variables that have the highest effect within each cluster to describe the distribution in space.

For all statistical analyses, p were considered significant at $\leq .05$, while p between .10 and .05 were considered as tendencies (Averós et al. 2010).

Results

Descriptive statistics

Table 4 shows the descriptive statistics of the explanatory variables. The majority of the cats were adult and of European breed. Even though for almost a quarter of them it was the first veterinary visit, almost half of them was familiar with pet carriers and car rides. The owners described as aggressive only 10% of the cats, and the frequency of bites/attempt to bite and scratching was also less than 10%. However, almost 25% of cats attempted to scratch and hissed. Half of the cats vocalised and 85% of them showed very large pupils. The most common profiles were indeed agonistic and frightened.

Table 5 shows the descriptive statistics of the physiological parameters. However, physiological parameters could often not be measured for all cats during each visit (e.g. respiration was not measured for several cats because they were sniffing when the respiration rate was being measured). The average RT was within the normal range, but there were a few cases of pyretic cats. RR was within the normal range but HR was higher than normal.

Univariate logistic regression

There was a positive association between mydriasis and HR (OR: 1.1, 95%CI: 1.0–1.1; $p = .018$) and RR (OR: 1.1, 95%CI: 1.0–1.2; $p = .040$), indicating that for each increase in HR and RR cats were more likely to show mydriasis.

Table 5. Descriptive statistics of rectal temperature (RT), respiratory rate (RR), and heart rate (HR) recorded in 95 cats during a veterinary visit.

Variable Name	Mean	Standard Deviation	Min	First Quartile	Median	Third Quartile	Max
HR (beats per minute, bpm)	156.3	30.8	100	130	150	196	220
RR (breaths per minute, bpm)	30.4	9.6	10	20	30	40	44
RT (°C)	38.7	0.6	37.1	38.4	39	39	39.9

There was an association between the position of the ears and the temperament, with non-aggressive cats more likely to show the alert ear behaviour than aggressive cats (OR: 4.5, 95%CI: 1.1–18.5; $p = .038$), whilst aggressive cats were five times more likely to show the ear flat back behaviour than non-aggressive cats (OR: 5.5, 95%CI: 1.4–21.8, $p = .015$). There was a tendency of a positive association between the ears flat back and the first visit ($p = .076$) and the non-European breed ($p = .063$).

Escape attempt was more likely to happen in young cats than adult ones (OR: 3.2, 95%CI: 1.1–9.4, $p = .030$), and tendencies were observed for first visit (OR: 2.6, 95%CI: 1.0–6.9, $p = .056$) and non-European cats compared to the European ones (OR: 3.3, 95%CI: 0.9–11.7, $p = .063$).

Scratching/attempt to scratch was more likely to be shown by elderly cats compared with adult ones (OR: 3.8, CI: 1.2–11.6, $p = .020$), when cats had lower HR and RR ($p = .015$ and $p = .024$, respectively). A tendency was also observed for the temperament, with aggressive cats being more likely to show scratching/attempt to scratch (OR: 3.7, 95%CI: 1.0–14.27, $p = .055$). A tendency was also observed between hard stare behaviour and cat temperament, with aggressive cats more likely to show hard stare behaviour (OR: 6.8, 95%CI: 1.0–47.1, $p = .051$).

Cats vocalised more when they were not familiar with pet carriers (OR: 3.0, 95%CI: 1.3–7.0, $p = .010$) and with travelling (OR: 3.5, 95%CI: 1.5–8.6, $p = .005$), and males vocalised more than females (OR: 7.4, 95%CI: 2.1–25.6, $p = .007$).

Cats which were familiar with travelling were more likely to hiss (OR: 4.7, 95%CI: 1.5–15.2, $p = .010$).

Piloerection was associated with gender ($p = .049$) (castrated males were more likely to show it than intact males or females; OR: 5.2, 95%CI: 1.2–22.3, $p = .025$), with increased RR ($p = .046$) and aggressive temperament ($p = .017$). Tendencies were also noticed between piloerection and age ($p = .054$) with elderly cats being more likely to show piloerection than young cats (OR: 5.6, 95%CI: 1.3–23.5, $p = .018$), and between piloerection and travelling, with cats not familiar with travelling being more likely to show piloerection (OR: 5.2, 95%CI: 1.3–20.3, $p = .082$).

A tendency was also noticed between tail lashing and temperament, with this behaviour being more likely to be shown by cats with an aggressive temperament (OR: 4.1, 95%CI: 1.0–16.4, $p = .051$).

No other significant associations or tendencies were found.

Univariate multifactorial logistic regression of total behavioural score

Age was a significant variation factor with young cats displaying higher probabilities of having high values of the total behavioural score when compared with adult individuals (OR= 1.4, 95%CI = 1.1–1.9, $p = .010$). Intact females had, on the whole, lower behavioural scores, while castrated males were more likely to be scored with high values when compared with intact females (OR= 1.7, 95%CI = 1.2–2.4, $p = .002$), neutered females (OR = 1.3, 95%CI = 0.9–1.7, $p = .083$) and intact males (OR= 1.3, 95%CI = 1.0–1.6, $p = .066$). Cats described by their owners as having an aggressive temperament were 1.5 times more likely to show high behavioural scores compared to non-aggressive subjects (OR: 1.5, 95%CI = 1.1–2.0, $p = .011$), and those that were not familiar with travelling were in general associated with higher behavioural scores (OR= 1.4, 95%CI = 1.0–1.9, $p = .050$). A tendency was also noticed for the first visit, with higher behavioural scores being more likely to be attributed to cats during their first veterinary visit (OR= 1.1, 95%CI = 0.9–1.5, $p = .083$).

Chi-square test

The profile given to each cat at the end of the visit was associated with age ($p = .038$; Figure 1) and gender ($p = .007$; Figure 2), but not with the breed, temperament, first visit, carrier and travelling, or reason. Young cats were associated with the anxious profile, adult cats were more often alert or agonistic than anxious or frightened, whilst elderly cats were associated with the frightened and irritated profile.

Female cats seemed to be more alert, while neutered females were more frightened. Male cats were more likely to be categorised as irritated or frightened.

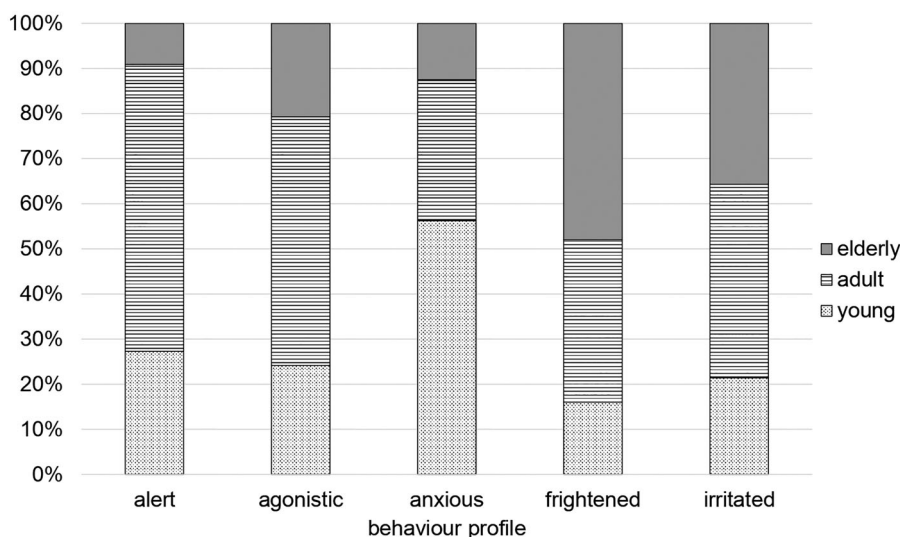


Figure 1. Association between the age and the profile of the cat subjected to a veterinary visit.

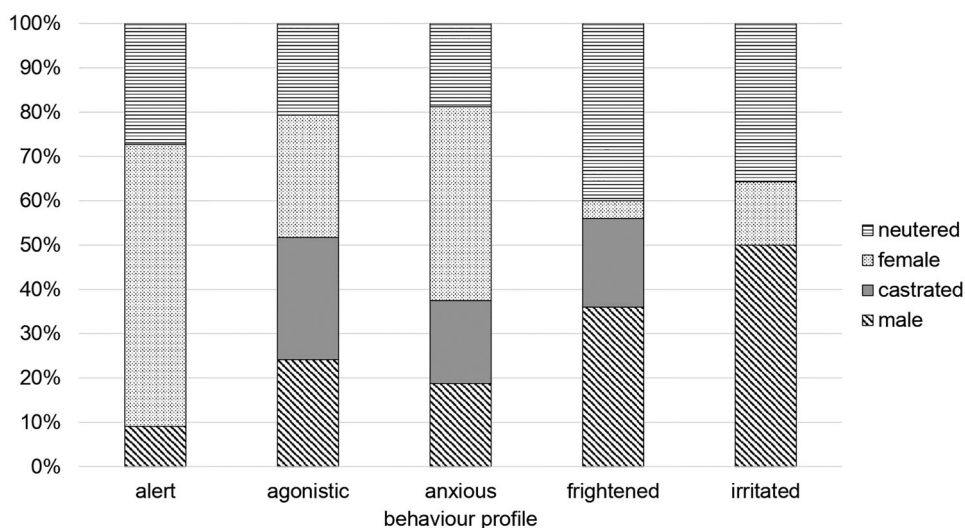


Figure 2. Association between the gender and the profile of the cat subjected to a veterinary visit.

MCA and HCPC

The top ten new dimensions identified by MCA explained 63.2% of the total variance. The two dimensions explaining the highest variance percentages were Dimension 1 (Dim1) and Dimension 2 (Dim2), accounting for 11.7% and 8.7% of the total variance, respectively (Supplementary Figure S1 and Figure 3). The contribution of the variable categories (in %) to the definition of the dimensions are reported in Supplementary Table S1. The variables contributing the most to Dim1 were behaviours like hissing (9.4), piloerection (7.5) and vocalisation (4.2), together with the aggressive temperament (9.0), the young age (4.3) and the profile frightened (4.4) and anxious (4.1). On the other hand, Dim2 was determined by the presence of slit pupils (15.9), the behaviour of crouching in the pet carrier (7.5), the ears pressed flat back (5.0). Dim1

differentiated cats labelled with a frightened profile from those labelled as anxious or alert, while the variables entering in Dim2 permitted differentiation of the animals categorised with an agonistic profile from those displaying behaviours related to frightened, anxious and alert profiles. The MCA results were useful to differentiate the profiles based on cat's age and gender, with intact females more frequently displaying an alert profile, adult males and elderly cats displaying an agonistic profile and young cats an anxious profile. Finally, travelling resulted to be linked to alert and anxious behaviours, and the cats that were not used to car rides were more prone to display behaviours related to the agonistic profile.

The HCPC analysis indicated that the observations can be explained by four clusters of variables (Figure 4), mainly characterised by the presence or absence of

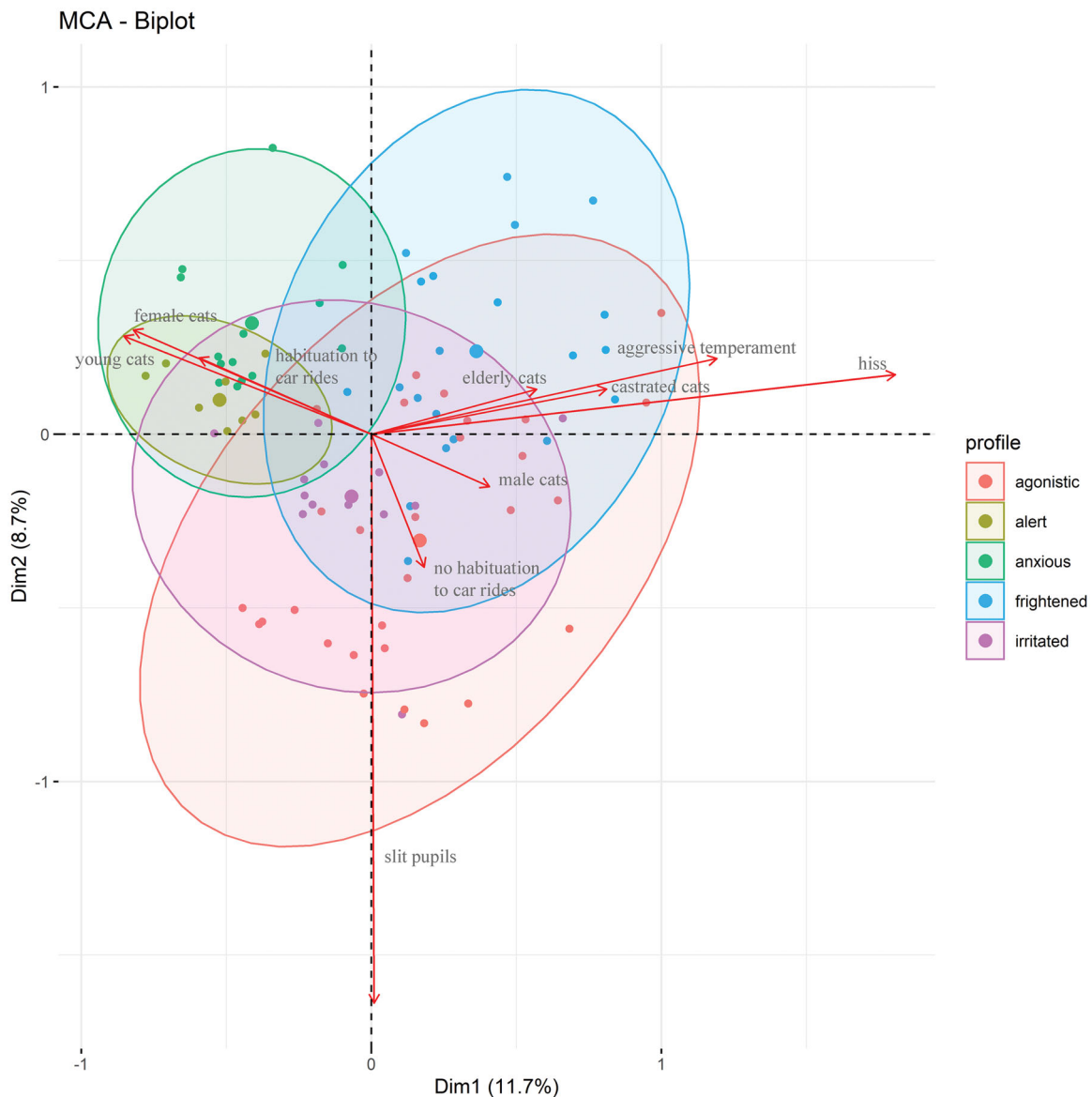


Figure 3. Multiple Correspondence Analysis (MCA) biplot. The plot reports the observations distinguished on the basis of the cats' profile and the vectors (red arrows) representing the variables with the greatest effect on the two new dimensions (Dim1 and Dim2).

mydriasis ($p = 1.3E-19$) and by the profile attributed to the cat at the end of the veterinary visit ($p = 2.01E-17$; [Supplementary Table S2](#)). The latter result proved that the profile attributed to each cat at the end of the visit had great importance in the representation of the variability of the cat behaviours. The [Supplementary tables S3, S4, S5 and S6](#) report the weights of the variables significantly explaining the variability inside each cluster. The variability observed in cluster 1 was mainly explained by alert and anxious (with the positive weights 5.12 and 4.44, respectively) and by agonistic (-4.31) profiles. On the other hand, the anxious and agonistic profiles were significant also for cluster 2 with the weights -2.50 and 4.44, respectively. Cluster 3 variability was related to irritated (2.48),

anxious (-2.94) and alert (-2.13) profiles, and, finally, the cats labelled with the frightened profile were represented by the cluster 4, since this variable had a weight of 5.13 for this cluster.

Discussion

This study provided information on the behaviour of cats during the veterinary examinations and the possible associations between cat behaviour and its reproductive status, age, temperament, the reason why it was brought to visit, its familiarity with pet carriers, car rides and veterinary visits. Our findings confirmed that a veterinary visit was a stressful and fearful event (Mariti et al. 2016, 2017), based on the recorded

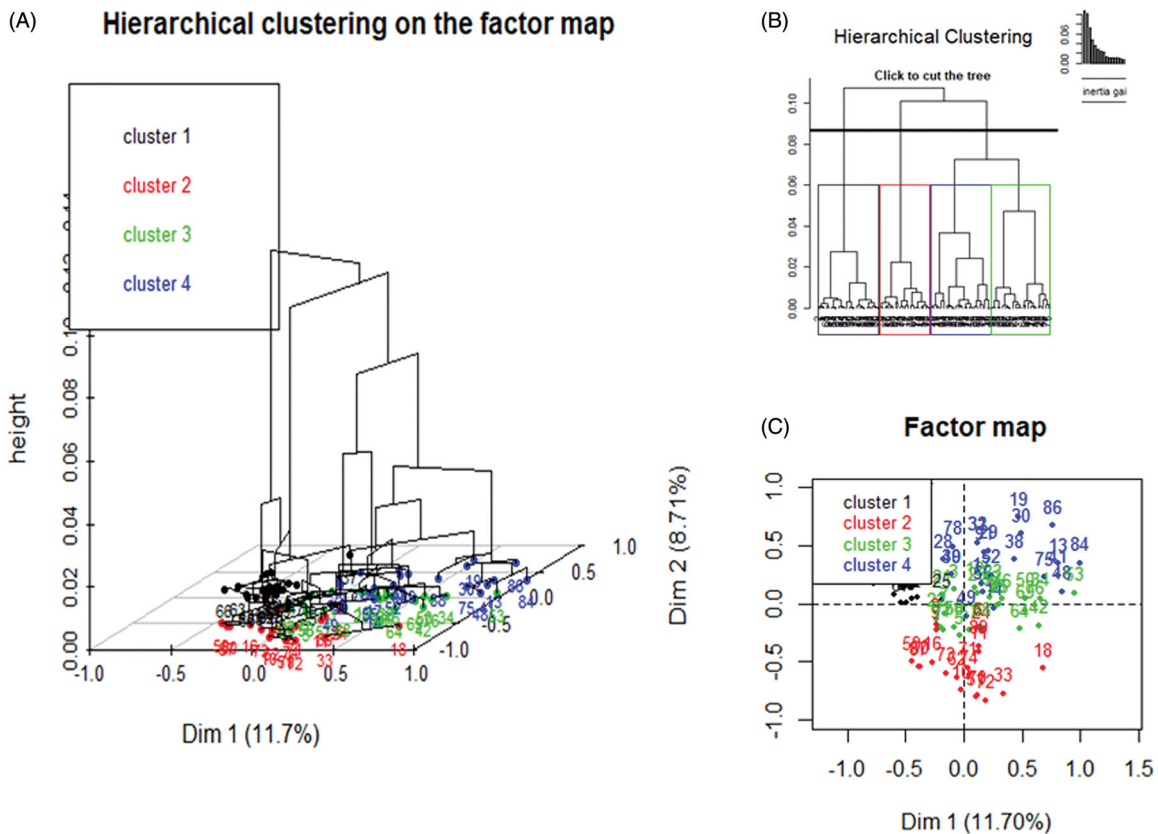


Figure 4. The results of the Hierarchical Clustering on Principal Components (HCPC) analysis. The 3D tree (A) displaying the hierarchy of the variables affecting the most the creation of the four clusters, the representation of the chosen height (0.06) to perform the cut of the hierarchical clustering tree (B), and the (Multiple Correspondence Analysis (MCA) biplot with the four clusters obtained from HCPC analysis (C).

physiological and behavioural responses typical of the hypothalamic-pituitary-adrenal axis activation (Carlstead et al. 1993). Cats showed indeed HR higher than normal and high frequency of mydriasis, and the cats at the first visit or young cats were more likely to try to escape. However, even if cats not familiar with pet carriers and car rides vocalised more and reported higher total behavioural score, cat familiar with car rides hissed more. So, our results do not completely support our hypothesis. It seems possible that our adult and elderly cats had developed negative associations with the veterinary visits and with all the events which anticipate them. Our findings suggest that particular attention should be paid to make sure that young cats do not have a bad experience during their first veterinary visit and whatever predicts it. A clearer association between the cat behaviour and intrinsic factors (age, gender and temperament) was instead found. Consequently, that information should always be collected before a clinical examination to reduce cat-aggressive behaviour-related injuries in veterinary staff. Our findings are helpful for cats' owners and

veterinarians to implement the preparation and management of a veterinary examination.

Cats displayed stress and fear-related physiological and behavioural responses during veterinary examination in our traditional veterinary clinic. This was expected and in line with the literature. Cats had higher physiological parameters (temperature, pulse, respiration and blood pressure) when examined in a veterinary clinic compared to their home (Quimby et al. 2011). However, in the latter study, cats' behaviour was not described, and the findings were not confirmed by Nibblett et al. (2015). These authors found a difference between cats visited at home and in a Cat Friendly Practice[®] (American Association of Feline Practitioners) only in blood glucose and in the hiding behaviour, but they failed to find any correlations between physiological and behavioural parameters. Our HR, RR and RT were in line with the value registered during veterinary cheques in the two studies mentioned before (Quimby et al. 2011; Nibblett et al., 2015). Only HR was higher than the normal range of 120–140 bpm (Detweiler and Erickson 2004)

and there was an association between HR and RR and mydriasis. HR and pupil dilation have been used as indicators of fear and distress in cats, as previously associated with amygdala and sympathoadrenal activation (Casey 2002; Moody et al. 2018). Consequently, the positive association we found is consistent with a physiological pattern of fear. Similarly, an association between mydriasis and RR was found by Moody et al. (2018) during full restraint. We, unfortunately, did not describe the type of restraining used during the examinations, and RR was taken once at the beginning of the examination. Even if physiological parameters are more objective, they may be difficult to collect, therefore, behavioural observation and mydriasis may be helpful for veterinarians to examine cats and interpret their clinical data. It is indeed well known that there is a nexus between the type of pathology and behaviour (Seibert and Landsberg 2008). Consequently, it is not surprisingly that our total behavioural score resulted associated with the reason for the visit, such as the finding that cats with gastroenteric problems showed a lower behavioural score. This could be due to the fact that those cats were debilitated, and their behavioural responses limited by the depressed demeanour. For these reasons, interpretation of cat behaviour is recommended for interpreting the clinical parameters affected by fear.

Increased vocalisation, side and back ear position, hiding, escape attempts and pupil dilatation are signs of fear and stress (Casey 2002; Moody et al. 2018). Almost all of our cats (85%) showed very dilated pupils (mydriasis) and one-third of the cats tried to escape. Nibblett et al. (2015) found that escape attempts were more frequent at home than in the clinic and that hiding was the only significant behaviour whose frequency was higher in the clinic than at home. Unfortunately, we did not record hiding, but only crouching in the container, which was shown only by 11% of the cats. However, the cats were removed from the container at the beginning of the visit, and this could have affected our data. On the contrary, vocalisation was shown by half of the cats and was more frequent in cats unfamiliar with pet carriers and car rides. These findings support the idea that habituation to pet carriers and car rides may reduce stress and fear in cats brought to a veterinary clinic. This provided that the training is carried out correctly and that the veterinary examination does not instead represent a negative experience capable of consolidating negative associations in the animal. Training methods helping associations between pet carriers and car rides with something positive have

been already suggested in the literature (Ghandour 2017; Houpt 2018; Pratsch et al. 2018). One of the most reproducible methods for reducing the pet's stress levels is for the pet to be acclimatised to the carrier and trained to use it at home. In the home environment, the carrier can become a 'safe' zone and/or a place that the pet associates with pleasant experiences such as feeding, so that, on the day of the travel, cats are less likely to be concerned about being inside the carrier (Ghandour 2017). However, we did not ask about the type of training applied by the owners, and we cannot be certain whether the examined cats had a positive or a negative association with pet carriers, car rides and veterinary clinics. Future studies will be carried out to clarify this point.

Behavioural responses were associated with intrinsic factors, such as age, gender and temperament. This is not surprising since behaviour is strongly affected by genetic, experience and environment (Houpt 2018). Young cats were more anxious and showed the highest total behavioural score, while elderly cats were irritated and more likely to show scratching, tail lashing and piloerection. A young cat may be scared of novelty, and this is why it would be extremely important to handle and manage young kittens at their first veterinary visit very gently, to avoid a bad association which may affect their behaviour in future veterinary examinations. This is in line with the guidelines of the American Association of Feline Practitioners. A bad association may explain the fact the adult cats were more likely to exhibit an agonistic profile and elderly cats a frightened or irritated one. Intact female cats seemed to be the category which was more associated with the alert and overall calm profile. This may be due to the presence of the progesterone, which has a calming effect (Hart and Eckstein 1997). However, it is not in line with the literature. Sex did not show an association with behavioural problems in cats, except that intact female were presented more frequently for aggression problems than neutered females in a study by Amat et al. (2009). While the associations with sex are unclear, many authors have found an association between behavioural characteristics and breed, coat type and eye colour (Wilhelmy et al. 2016). We found only a tendency between ears flat back and non-European breed, but our non-European cats were only 13% of the examined cats and of a variety of pure breeds. We did not register the colour, which has been proposed as an easy indicator for veterinarians (Delgado et al. 2012). However, our initial question to the owners on the temperament of the cats seems to be useful. The reported

aggressive temperament resulted indeed associated with the total behavioural score. Cats with an aggressive temperament were more likely to show piloerection, ears flat back, tail lashing, scratching and attempting to scratch. MCA also demonstrated a clear separation of those parameters in dimension 1. Consequently, asking about the temperament may be useful to reduce the animal-related injury to veterinary staff, which are considered occupational injuries (Gabel and Gerberich 2002; McGreevy et al. 2007). The MCA and HCPC analysis also showed a strong relationship between the behaviour and the profiles we used. This was expected since the profile was chosen based on the behavioural observation. However, our statistical analysis validated our profiles. Give a profile to a cat at the end of a visit may be easier than filling in an ethogram, consequently, we would like to recommend adding a profile to the clinical records. Knowing the type of profile may help managing future visits of that cat.

Transport is stressful for animals (Padalino 2015). Cats' owners reported that cats vocalise, defaecate and vomit during car rides, and owners' perception of their cats' stress level during transportation and veterinary examination has been identified as a barrier to cats receiving preventive veterinary care (van Haaften et al. 2017). This is why drugs and training have been suggested to reduce transport stress (Rose et al. 2010; Landsberg et al. 2013; Overall 2013). Our cats did not receive any drugs or pheromones before going to the clinics, and this could explain why cats which were not familiar with pet carriers and car rides kept vocalising more during the examination. Drugs and pheromones have been suggested also to reduce bad associations with the rides and the veterinary examinations (Rose et al. 2010). If the cats had a positive or neutral experience during their first visit, they may have had less stress during subsequent visits (van Haaften et al. 2017). Based on our findings, we can infer that cats at their first visit have a higher level of stress and fear, indicating that cats habituate to veterinary visits to some extent. This should be taken into account when interpreting our findings.

Our results need to be interpreted with caution also because the study was limited by several factors. The first limitation was the type of behavioural sampling, we used a 1/0 sampling method and consequently, we did not have the overall frequency and duration of the behavioural events and states. We also did not record the sequence of the behaviour, so in some cases, we had to arbitrarily delete some mutually exclusive behaviours. Moreover, we did not record the exact duration of

the visit so we could not determine whether longer visits caused a higher total behavioural score. We recorded the data only in one clinic, minimising the effects of the different management and handling procedure used by the veterinarians. However, this implies that our data need to be repeated on a larger population of cats examined in a large sample size of veterinary hospitals. We did not ask about training practices used to familiarise cats with pet carriers and car rides, and this could have biased the associations we found. Finally, videotaping the visits would have been useful to better assess and annotate the cat's behaviours. Anyway, it was not possible to record the cats without including the owner's and vet images, and thus no videotapes were recorded due to privacy reasons. Notwithstanding those limitations, this manuscript has value due to the large sample size ($n=95$) and it has increased our knowledge of the behavioural responses of cats during veterinary examination not conducted in a Cat Friendly Practice[®] (American Association of Feline Practitioners) or Fear Free[®] certificated clinic (<https://fearfreepets.com/>), confirming that cats experienced distress and fear during those types of visits, and consequently, management to reduce stress and fear in feline patients should be applied (Nibblet et al. 2014). Our findings may be useful to improve guidelines in the conduction of veterinary examinations enhancing feline welfare and reducing the risk of injuries in veterinary staff.

Conclusions

This study documented behavioural responses of 95 cats visits to a traditional veterinary clinic. The results confirmed that veterinary examinations were perceived as stressful and fearful events leading to tachycardia, mydriasis and often to behaviour and body language related to fear and aggression. Age and gender were associated with the cats' behaviour confirming the nexus between behaviour, genetic and environmental effects. Familiarity with pet carriers and car rides seems to minimise stress and fear behavioural responses, but those results need to be ascertained in further studies, collecting more information on the type of training used for travelling and past experience of the cats during car rides and clinical examinations.

Ethical Approval

This prospective cohort study was carried out with the approval of the animal ethics committee of the

Department of veterinary medicine, the University of Bari, Italy (CESA n. 11/2020).

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No potential conflict of interest was reported by the author(s).

Author contributions

Conceptualisation, A.T. and A.C.; Methodology, A.T. and A.C.; Formal Analysis, M.Z. and B.P.; Data Curation, M.Z. and B.P.; Writing-Original Draft Preparation, A.T., M.Z. and B.P.; Writing-Review & Editing, B.P.; Supervision, B.P.; Funding Acquisition, A.T.

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