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Inter-Observer Agreement And Sensitivity To Climatic Conditions In Sheltered Dogs' Welfare Evaluation Performed With Welfare Assessment Protocol (Shelter Quality Protocol)

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1 INTER-OBSERVER AGREEMENT AND SENSITIVITY TO CLIMATIC CONDITIONS IN

2 SHELTERED DOGS' WELFARE EVALUATION PERFORMED WITH WELFARE

3 ASSESSMENT PROTOCOL (SHELTER QUALITY PROTOCOL)

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39 Abstract

The Shelter Quality Protocol (SQP) is a concise and easily implemented tool for assessing dog 40 welfare and to identify critical aspects of the shelter environment. A first version of the protocol has 41 been modified in order to improve its performance. The aim of this study was to evaluate the 42 reliability of the measures included in the second version of the protocol (SQP2) by testing the 43 inter-observer agreement between two independent assessors. We evaluated the sensitivity of 44 animal-based measures in detecting the shelter dogs' welfare outputs during two different seasons. 45 Ten Italian shelters were assessed contemporaneously by two assessors to determine the reliability 46 of SQP2 measures. Inter-observer agreement was evaluated using the Cohen's Kappa for qualitative 47 variables and Pearson' correlation for quantitative variables. The SQP2 was also applied twice 48 49 (January and August) by the same observer in five Italian shelters to evaluate the sensitivity of the protocol to seasonal condition changes. The quantitative variables, "Number of animals 50 shivering/huddling" and "Number of animals panting" were analyzed by Wilcoxon test. Credible 51 52 intervals (95%) were calculated using a beta distribution for qualitative variables: "Body condition", "Skin condition", "Dog cleanliness", "Signs of diarrhea", "Coughing", and "Lameness". 53 The level of agreement between the two observers on the qualitative variables such as body 54 condition, lameness, skin condition, was quite high, ranging from substantial (0.61-0.80) to almost 55 perfect (0.81-0.99). Inter-observer agreement was also significant with Pearson correlation 56 coefficients ranging from 0.51 to 0.92 (e.g.; curious = 0.74; sociable = 0.83; barking level = 0.61). 57 "Number of animals panting" and "Signs of diarrhea" showed a significantly difference between the 58 assessments (p<0.05). Animals with lameness, coughing and inadequate body condition increased 59 60 in the winter season whereas the skin lesions increased during the summer, but not significantly. The behaviors of shivering/huddling were observed too infrequently to be meaningfully analyzed. 61 Consistent inter-observer agreement exists in assessing dogs' welfare using the SQP2 confirming 62 63 the reliability of the measures included in the protocol. The SQP2 shows potential in detecting

changes in dogs' welfare outputs due to different climatic conditions. Further investigations are
required to confirm the sensitivity of selected measures to different seasons.

- 66 Keywords: dog; animal welfare assessment; shelter; protocol; reliability; sensitivity
- 67

68 **1. Introduction**

It is widely recognized that the shelter environment negatively affects animal welfare and poses a 69 challenge to most dogs. The main challenges include the unfamiliar housing systems, different daily 70 routines, changes in feeding regimen and type of food, unfamiliar sounds, smells and sights, social 71 deprivation, presence of several unfamiliar animals and humans, and the absence of an attachment 72 figure (Moesta et al., 2015; Tynes et al., 2015). Several studies have reported that the stress 73 associated with shelter environment can contribute to onset of behavioral problems, such as stress-74 related aggression, abnormal or repetitive behaviors, anxiety and fear-related disorders (Tuber, 75 1999, Beerda et al., 2000; Hennessy et al., 2001; Hiby et al. 2006; Dalla Villa et al., 2013; Titulaer 76 et al., 2013). However, the behavioral responses of the dogs can vary depending on the stressors 77 78 (acute or chronic; physiological or psychological) and individual variability (e.g. genetics, age, early life experiences, and the success or failure of previous responses to stress) (Horowitz, 2004; 79 80 Moesta et al., 2015). Moreover, the experience of dogs in shelters is of concern, not only in terms of animal welfare, but also for its potential effect on the likelihood of adoption (Diesel et al. 2013; 81 Duffy et al, 2014). In the worst case, dogs remain in the shelters for the remainder of their life or, in 82 the countries where "no-kill" policy is not in force (e.g., USA, UK some regions of Spain), they are 83 euthanized if they are not adopted (Moesta et al., 2015). In Italy, however, euthanizing dogs is 84 forbidden by the law unless they are seriously ill, incurable or proven dangerous (Italian law 85 281/1991). The dogs can be hosted in long-stay facilities until the day of the adoption. Improving 86 the welfare of shelter dogs must be considered a primary goal of rehoming centers. One way to 87

improve welfare is to reduce the time dogs spend in the shelter environment. This could be achieved
through successful adoptions and a decreased return rate (Posage et al., 1998; Diesel et al., 2008;
Luescher and Medlock, 2009; Braun, 2011; Reid and Collins; 2015). The detrimental effects of a
shelter environment can be mitigated through providing adequate housing and management which
meet the dogs' ethological needs, and ensuring the highest quality of care in the case of long-term
sheltering (Miller and Zawistowski, 2015).

The Shelter Quality Protocol (SQP) was developed in response to the issues related to long-term 94 sheltering and it fills the existing gap in the assessment of shelter dog welfare. This gap is due, in 95 part, to the lack of and/or variation in regulatory frameworks defining minimum requirements for 96 shelters (Barnard et al., 2016). The SQP was designed to be concise and easy to implement in 97 assessing dog welfare (Barnard et al., 2016). This protocol was inspired by the Welfare Quality® 98 approach. In particular, it was built around the four principles of good feeding, good housing, good 99 100 health and appropriate behavior. Each one of these principles are composed of different welfare criteria which in turn include different welfare measures (Welfare Quality®, 2009). Since welfare is 101 102 the outcome of multi-factorial effects, multiple variables need to be considered when applying the 103 protocol (Sherman, 2010; Barnard et al., 2016). For example, the criterion "Absence of prolonged hunger" is composed of welfare measures: "Body condition" and "Feeding". The latter measure 104 includes "Type of diet", "Presence of special diets" and "Feeding regime". The measures were 105 selected to assess specific welfare criteria, reflecting management procedures (management-based 106 measures), housing environment (resource-based measures) and direct welfare outcomes (animal-107 based measures). The animal-based measures are considered the best indicator of animal welfare 108 because they give a direct reflection on animal welfare state (EFSA, 2012; Kiddie and Collins, 109 2014). However, in some cases, resource- and management-based measures were maintained 110 because of the valuable information they provide to complement the animal-based measures 111 (Veisser et al., 2011). The SOP provides three different levels of assessment: i) measures taken at 112 shelter level, which encompass all management-based measures; ii) measures taken at pen level (113

114	both resource- and animal-based measures) assessed by observing a random sample of pens and all
115	animals confined within; iii) measures taken at the individual level: all animal-based, assessed by
116	observing a sub-sample of dogs housed in the pre-selected sample of pens.
117	The approach of SQP can be considered innovative for companion animal welfare assessment.
118	Although the protocol was validated through field testing, which demonstrated its feasibility and
119	reliability, the SQP was modified in light of subsequent field application and feedback (Barnard et
120	al., 2016). The refined version of the Shelter Quality Protocol (SQP2) remained similar in structure
121	to the first version (SQP). The SQP2 was built following the Welfare Quality® principles (4) and
122	criteria (12) (Table 1).
123	The aim of this study was to assess the reliability of SQP2 measures. This was achieved by testing
124	the inter-observer agreement between two assessors who evaluated a sample of ten dog shelter
125	which were long-term confinement shelters. Reliability indicates the reproducibility of
126	measurements, in particular it is the degree to which a measure is free from errors and will therefore
127	yield the same results when repeated (Taylor and Mills, 2006; Martin and Bateson, 2007;
128	Thanasegaran, 2009). In addition, the SQP2 was tested in five shelters to evaluate the sensitivity of
129	selected animal-based measures in detecting the shelter dogs' outputs related to seasonal conditions.
130	Sensitivity concerns the ability of a tool (e.g., a protocol) or measurement to detect small but
131	important changes (Martin and Bateson, 2007).

132

2. Material and Methods

133 2.1 Shelter Quality protocol-second version (SQP2)

In a previous study by Barnard et al. (2016), the SQP was proven to be a valid, reliable and practical tool for assessing dog welfare in long-term shelters and for identifying critical aspects and welfare risks in shelter management and environment. In order to improve the animal welfare measurements and increase the applicability to persons with varying backgrounds (e.g., veterinarians and shelter

138	operators) (Taylor and Mills, 2006; Barnard et al., 2016) the SQP was reviewed and refined using
139	feedback obtained from its dissemination and field application. This resulted in the Shelter Quality
140	Protocol-second version (SQP2). The SQP2 contains 23 welfare measures, which mainly involve
141	animal-based measures as these allow for direct information on the welfare state of animals. The
142	welfare indicators within the protocol were selected to assess the above-mentioned principle and
143	criteria identified by Welfare Animal® Consortium (Blokhuis et al., 2010) (Table 1).
144	The refinements of the SQP included both the elimination and introduction of specific variables to
145	improve tool performance; changes in the level of assessment (i.e., from shelter to pen level or from
146	individual to pen level) to obtain more accurate information and reduce assessment time; and,
147	finally, the modification of the measurement approach to simplify the data collection (e.g.,
148	defensive and offensive aggression was merged in a single behavioral category). The refinements
149	are set out below in detail.
150	The variables "Nasal discharge" and "Dyspnea" included in the SQP were eliminated due to the low
151	prevalence (below 1 per cent) of expression resulting from previous on-field assessment of 29
152	shelters. "Morbidity" was also excluded due to the difficulty in collecting this information, shelter
153	managers not always are able to provide this information (Barnard et al., 2016).
154	The level of assessment of "Signs of diarrhea", "Coughing" and "Evidence of pain" was altered
155	from the individual level to pen level in order to obtain a more accurate estimate. The assessor had
156	to record these variables by observing all the animals in the pen instead of taking a sample of
157	selected dogs.
158	In the SQP2 welfare measures "Barking level" and "Emotional state" were assessed at pen level
159	instead of the shelter level. The assessment of shelter dog emotional state was performed using a
160	Qualitative Behavioural Assessment (QBA). QBA is a method which focuses on the observation of
161	the whole animal and characterizes and quantifies the animal's dynamic demeanor as an expressive
162	body language using descriptors such as "sociable", "aggressive" or "anxious" et cetera
163	(Wemelsfelder, 2000). Arena et al. (2017), through a scientific approach (Free-Choice-Profiling

methodology) developed a list of terms specifically focused on shelter dogs emotional state. Thislist was included in SQP2.

166 To obtain information about shelter turnover, the following variables were included: "Number of

dogs entered in shelter", "Number of adopted dogs", "Number of dogs returned after adoption",

168 "Number of dogs returned to owner".

169 In order to obtain information about the promotion of human-animal relationship in the shelter the

variables "Presence of qualified personnel for dog training", "Presence of qualified personnel for

behavioral rehabilitation" were added. There is evidence that in shelters basic dog training can help

the development of mental activities and constitute the basis for an adequate human-animal bond,

173 which is important for successful adoptions. Similarly, dog behavioral rehabilitation facilitates the

174 human-animal relationship, improves dog welfare and increases the likelihood of adoption (Taylor

and Mills, 2007; Luescher and Medlock, 2009).

176 The variable "Shelter from adverse weather conditions" (sun, wind, rain) was introduced in the

177 SQP2 to replace the resourced-based measures "indoor/outdoor area". This addition alleviated

issues which arose during the on-field assessment using SQP for the assessment of thermal comfort

179 (criterion "Good housing") due to the different definitions of outdoor/indoor area established by

180 each Italian regional law.

The last refinement of SQP merged defensive and offensive aggression into a single category to
assess the reaction of dogs toward unfamiliar people in order to facilitate the interpretation of dogs'
behaviors (Barnard et al., 2016). These refinements are summarized in Table 2.

Before the on-field application, the two assessors were familiarized with SQP2 through teachingmaterials (videos and photos).

186 2.2 Inter-observer agreement assessment

An inter-observer agreement was performed to assess the reliability of the protocol measures after
 refinement (SQP2). Ten Italian long-term shelters were evaluated by two different assessors

simultaneously and independently. The two assessors were female, aged between 30 and 40 years, 189 both veterinarians with specialization in applied ethology and animal welfare. Both assessors had 190 previous experience working with dogs and were made familiar with the SQP2 by training in field 191 and by video and photo support. Shelters were selected on the basis of the following inclusion 192 criteria: long-stay facility and manager availability to take part in the study. The shelters were 193 located in four regions of North and Central Italy: Trentino (1), Veneto (2), Emilia Romagna (6), 194 and Marche (1). The assessment was carried out according to the methodological procedures 195 described in the SQP2 (Shelter Quality protocol, 2017) 196 The dogs were assessed while housed in their home pen. The pens were selected using a shelter map 197 and on the basis of the number of dogs housed in each pen (taking sample size into account). The 198 sample size depended on the total number of dogs housed in the shelter at the time of visit (Table 199 3). The selected pens covered the different facilities. The sample of animals assessed at individual 200 201 level only included dogs over 6 months of age and those who had been housed in the shelter for 2 months or more. A maximum of three dogs per pen were assessed at individual level. The shelter 202 203 assessment was carried out in a single day.

As previously underlined, the measures were taken according to 3 scoring levels: shelter level (the shelter was evaluated as a unit), pen level (each selected pen was evaluated as a unit and all animals confined were observed irrespective of the total number of animals) and at the individual level (each selected animal was evaluated as a unit). Except the management section (measures at shelter level) that consisted of an interview with the shelter manager, the whole assessment was carried out independently by the two assessors.

The assessment was carried out first at the pen level. The assessor stood in front of the pen, two meters from the fence and without interacting with animals (unless this was required by the protocol, e.g., short test to assess reaction towards human), recording the measures at pen level. The individual animal-based measures were then recorded. Among these measures, a short behavioural test was carried out to assess the dogs' reactions towards unfamiliar people. The test

was divided in two steps in order to record the dogs' reaction. First, the assessor approached the fence, standing in front of the pen and ignored the dog for 30 seconds. Second, the assessor crouched talking gently to the dog for 30 seconds. Finally, the assessor recorded the emotional state of dogs in the pen by filling in the Emotional State Profile sheet.

After assessing the first pen, the assessor moved on to the next one, following the same procedure.

220 The assessment ended when the last pen was assessed (Shelter Quality, 2017).

Inter-observer agreement was evaluated using the Cohen's Kappa for qualitative variables; these variables were all categorical. The Pearson' correlation was used for quantitative variables which were discrete and continuous. Level of significance was set respectively at $\alpha \le 0.0020$ and $\alpha \le$ 0.0025, after applying the Bonferroni correction. For all analyses, z scores and p values were also computed to indicate whether agreement was more than could be expected by chance alone (Cohen, 1968). Table 4 summarizes the different variables with their score system.

227

228 2.3 Seasonal sensitivity protocol assessment

To better define the sensitivity of SQP2 to seasonal changes, five Italian long-term shelters wereassessed during winter and summer.

The SQP2 was applied twice (January and August) by the same assessor in five selected shelters located in three regions of Central Italy: Marche (1), Abruzzi (3) and Molise (1). Shelter inclusion criteria were the same as those used for the inter-observer agreement assessment. The assessments were carried out following the same procedure described above. During the assessments,

temperature and humidity were recorded.

A random selection of dogs were used for each assessment because the shelter population and the

237 location of dogs in their pens could change in time. This sampling approach reduced possible bias

in the results by limiting the likelihood that the assessor could remember the scoring of the dogs

recorded during the first assessment (Barnard et al., 2016).

240	Since animal welfare may be affected differently between seasons, eight variables potentially
241	sensitive to changes in seasonal conditions were included in the analysis. These measures were
242	selected because animal-based measures can give direct information on dogs' output. They were
243	assessed according to the SQP2.
244	The variables selected were either quantitative - i.e., "Number of animals shivering/huddling",
245	"Number of animals panting" - or qualitative - i.e., "Body condition", "Skin condition", "Dog
246	cleanliness", "Signs of diarrhea", "Coughing" and "Lameness". The quantitative variables were
247	analyzed by Wilcoxon test. Credible intervals (CI) (95%) were calculated using a beta distribution
248	for qualitative variables.
249	The authors hypothesized that the likelihood of observing animals panting, with diarrhea, or with
250	skin lesions, would increase during summer season, whereas the likelihood of observing animals
251	shivering/huddling, coughing, with inadequate body condition, with lameness, and with dirty coat

would increase in the winter season. Statistical analyses were carried out using R V.2.15.3.

253 **3. Results**

254 3.1 Inter-observer agreement

255 For the inter-observer agreement study, 222 pens and 710 dogs, living in the same pens, were assessed over a population of 847 dogs hosted in 406 pens. A subsample of 365 dogs were selected. 256 257 After analyzing the qualitative variables, the Cohen's Kappa analysis showed a high level of agreement between the two observers, ranging from substantial (0.61-0.80) to almost perfect (0.81-258 259 0.99) for the majority of variables. As could be expected, perfect agreement was obtained for the 260 variable of "Type of drinkers" (k= 1). The measures of "Shelter from rain" (k= 0.89), "Age class" (k= 0.89), "Shelter from strong wind" (k= 0.88), "Skin condition" (k= 0.84) and "Lameness" (k= 261 262 0.82) obtained an almost perfect agreement. On the contrary, the variable "Active repetitive 263 behaviors" showed a fair agreement (k=0.30). It was not possible to calculate the correlation for the

variables of "Other compulsive behaviors", "Air circulation", "Shelter from excessive sun",

265 "Evidence of pain", "Coughing", because of the lack of variability in the data (Table 5).

- Analyzing the quantitative variables, the agreement among the two assessors was also significant
- with Pearson correlation coefficients ranging from 0.51 to 0.92. In particular, the number of animals

subdivided into two categories ("N° of animals ≤ 20 kg": 0.92; "N° of animals > 20kg": 0.91)

- obtained high agreement (Table 5).
- 270 Level of agreement between the two assessors on the most QBA adjectives (9/13) was ranging from

substantial (0.61-0.80) to almost perfect (0.81-0.99) whereas the agreement of remaining adjectives

272 (4/12) was scored as fair (ranging from 0.41 to 0.60). The variables "N° of animals

shivering/huddling" achieved the perfect agreement. For the variable "N° of animals panting" the correlation analysis could not be carried out due to the high homogeneity of the data. All results are summarized in Table 5. All P values were significant (P<0.001).

276

277 3.2 Seasonal sensitivity protocol assessment

For climatic sensitivity protocol assessment, 244 pens and 612 dogs (304 in summer and 308 in
winter), living in the same pens, were assessed over a mean population of 935 dogs hosted in 398

280 pens. A subsample of 505 dogs were selected (254 in summer; 251 in winter). The recorded

temperature and humidity during the different assessments are summarized in Table 6.

In summer 20% (60/304) of dogs showed panting whereas no dogs showed this behavior in winter

283 (0/308). Number of animals panting significantly increased during the summer season compared to

- winter season (Wilcoxon, p=0.0001). In winter season only 1% (3/308) of dogs showed
- shivering/huddling. No dogs showed this behavior in the summer (0/304). The behaviors of

shivering/huddling were observed too infrequently to be meaningfully analyzed.

287 The Beta distribution showed that the observation of "Signs of diarrhea" was statistically significant

in summer season (Summer: percentage of observations = 21.4%, CI: 0.15-0.29; Winter: percentage

289	of observations 7.6%, CI: 0.04-0.13). Although no statistically significant differences were
290	detected, "Body condition" (Summer: percentage of observations = 9,2%, CI: 0.06-0.13; Winter:
291	percentage of observations 12.4%, CI: 0.09-0.17) "Coughing" Summer: percentage of observations
292	= 0.8%%, CI: 0.002-0.048; Winter: percentage of observations 3.2%, CI: 0.01-0.07), "Skin
293	condition" (Summer: percentage of observations = 8.3%, CI: 0.05-0.12; Winter: percentage of
294	observations 7.3%, CI: 0.04-0.11) and "Lameness" (Summer: percentage of observations = 2.4%,
295	CI: 0.01-0.05; Winter: percentage of observations 4.1%, CI: 0.02-0.07) seemed to support the
296	hypothesis of the authors showing results in the expected direction. The observations of animals
297	with lameness, coughing and inadequate body condition increased in the winter season whereas the
298	skin lesions increased during the summer. "Dog cleanliness" (Summer: percentage of observations
299	= 16,7%, CI: 0.12-0.22; Winter: percentage of observations 12.9%, CI: 0.09-0.17) didn't show
300	statistically significance difference between the two assessments and the result was opposite to the
301	expected direction.

4. Discussion

SQP was considered an innovative approach to companion animal welfare assessment, particularly for long-term shelter dogs. Its validity, reliability and feasibility were proven with a previous study by Barnard et al. (2016). Since the feedback obtained from its application on field and its dissemination, a refinement of the protocol aimed to improve its performance in assessing dogs' welfare. The modifications made in SQP permitted the development of the second version of the protocol (SQP2).

The consistent level of agreement obtained between two assessors evaluating a sample of ten shelters highlighted that the changes made on SQP didn't affect the tool performance and confirmed the reliability of measures in the canine welfare assessment. Moreover, since the stressors may differ between seasons, the climatic sensitivity of some animal-based measures suggested the potential usefulness of the SQP2 in assessing changes in dogs' welfare outputs.

On the basis of the results, some considerations of the SQP2 can be made. In particular, the variable 314 "Signs of diarrhea", that in SQP2 is assessed at pen level instead of individual level, showed 315 substantial agreement and therefore allowed the assessment to be simplified. When group housed, 316 recording signs of diarrhea by considering individual animals does not allow to gain a proper 317 estimate because the presence of diarrhea cannot be associated to an individual dog. The presence 318 of liquid manure in group housing pens allows the detection of animals with potential clinical 319 problems (e.g., enteric disorders) and, consequently, the identification of which individual is 320 affected. This observation allows team member to carry out clinical examinations and treatment 321 (Sokolow et al., 2005; Newbury et al., 2010). This animal-based measure was also significantly 322 sensitive to climatic changes. In this study, the prevalence of diarrhea was found to be higher in the 323 summer; this could depend on the seasonality of gastrointestinal diseases such as intestinal parasites 324 (McCarthy and Moore, 2000; Fontanarrosa et al., 2006). 325

326 Although the other animal-based measures which were analyzed didn't show statistically

327 significance in the seasonal comparison, results were consistent with the hypothesis of the authors.

328 Outbreaks of coughing and lameness may be increased by cold temperatures and high humidity. On

329 the other hand, the favorable environmental condition due to summer conditions can facilitate

ectoparasities' presence which, in turn, could cause the outbreak of skin problems in animals

331 (Altizer et al., 2006). To better understand the seasonality of clinical conditions in shelter's dogs,

332 further investigations are required.

Issues highlighted through the previous application and dissemination of SQP included the
challenges in assessing pen adequacy when both indoor and outdoor areas were present. The Italian
national framework law 281/1991 on companion animals and stray dog prevention does not provide
standards for the managing and keeping of dogs in shelters. Instead, this is defined at regional level.
Therefore, generating high variability in shelters nationally. Assessing such different housing
conditions was therefore challenging. This aspect was addressed in SQP2 by modifying the
assessment of the housing adequacy to encompass the ability of the shelters to house dogs from

adverse weather conditions. After this refinement, no difficulties were reported during the on-fieldapplication of SQP2, as demonstrated by the high level of agreement.

Moreover, maintenance of body temperature is essential for positive animal welfare. Therefore, 342 each dog housed in the shelter must be provided with adequate thermal comfort at all times of year. 343 Seasonal variation in conditions, such as low or high temperatures, may increase stress (Miller and 344 Zawistoski, 2015). The SQP2 allows the assessment of changes in dog welfare due to these 345 different seasonal conditions. In particular, the measure "Number of animals panting" was proved 346 to be sensitive to seasonal changes, increasing during hot seasons. In contrast, the relevance of the 347 measure "Number of animals shivering/huddling" remains still unexplored due to the low 348 prevalence of animals showing these behaviors during the cold season. Further research should 349 include a higher number of individuals to be assessed in winter season to confirm its relevance in 350 canine welfare assessment. 351

Panting as well as shivering and huddling with pen-mates are physiological responses shown by 352 dogs in order to cope with extreme temperatures and can be considered an indication of thermal 353 354 discomfort. For this reason, the presence of animals showing these behaviors can highlight inadequate housing and/or management which should be corrected accordingly (Rooney et al., 355 2009). Although animal age, breed and overall health status can affect an animal's tolerance of 356 temperature, generally the range from 20°C (68°F) to 30°C (86°F) of environmental temperature is 357 considered the "thermoneutral zone". In this range the dog is able to maintain normal body 358 temperature without a change in metabolic rate (National Research Council, 2006). United States 359 Department of Agriculture (2013) suggested the indoor facilities' temperatures must never fall 360 below 7.2°C (45°F) or rise above 29.5°C (85°F) for more than 4 consecutive hours. 361

362 The consistent agreement obtained in scoring QBA descriptors (anxious, relaxed, aggressive,

363 playful etc.) at pen level confirms that the fixed list of terms included in SQP2 allows a reliable

assessment of dogs' emotional experience in a shelter environment (Walker et al., 2016; Arena et

365 al., 2017).

367 To have good welfare, shelter dogs, such as all domesticated animals, should experience more positive (e.g., pleasure, happiness) than negative (e.g., fear, pain) emotions (Boissy et al., 2007). 368 The richness of the qualitative terms used gives to the assessment the power to address dynamic 369 aspects of welfare including subtle important differentiations, such as between "relaxation" and 370 "depression" or between positive and negative excitement ("excited" vs. "nervous"). From a whole-371 animal welfare perspective, the aim is to capture larger patterns of expression and their context 372 through a large range of terms. However, it has been demonstrated that training significantly 373 improves inter-observer agreement levels ensuring both the interpretation of terms and the use of 374 375 the QBA scale (Minero et al., 2015). "Barking level" obtained a moderate agreement. Excessive vocalization may be a sign of 376 frustration, distress or boredom (Rooney et al., 2009). The presence of subjects who vocalize 377 378 excessively might also have a detrimental impact on the other dogs housed in the pen (Petak, 2013). Moreover, high noise levels in dog shelters may cause hearing damage and public disturbance 379 380 (Beelsey and Mills, 2010). For this reason, assessing barking level could indicate that acoustic safety and noise mitigation strategies are required. Such strategies may include removing the 381 subject from the group, controlling visitors' access in the pens' area or building noise abatement 382

383facilities (Coppola et al., 2006; Scheifele et al., 2012).

The only measure which showed a low level of agreement was "Active-repetitive behaviors". In the 384 scientific community, there is controversy about the definition and the meaning of this behavioral 385 category. The terms "repetitive behaviors" and "stereotypies" are often used interchangeably. 386 Stereotypies are defined as repetitive and unvarying behaviors without apparent goal or function 387 (Mason and Latham, 2004). Some studies interpret repetitive behaviors in sheltered dogs as 388 389 indicators of compromised welfare, which may be related to stress and frustration or to confinement environments (Hetts et al., 1992; Beerda et al., 1999; Beerda et al., 2000). Their presence is usually 390 observed in association with sub-optimal environmental conditions (Denham et al., 2014; Mason, 391

1991), although this also depends on individual variability (Denham et al., 2014; Overall, 2013). 392 Mason and Latham (2004) underline that repetitive behaviors can be an individuals' strategy to 393 cope with stress and can be correlated with good or neutral welfare. The current results from the 394 measure "Active-repetitive behaviors" highlighted these problems with interpreting the occurrence 395 of these behaviors. Improving the accuracy of the definitions used in the protocol could improve the 396 reliability of this measurement. The reliability of the recording may be influenced by the clarity of 397 definition of behavioral category or measurement (Kiddie and Collins, 2014). For each possible 398 repetitive behavior shown by sheltered dogs it could be useful to specify, in addition to the 399 definition of the behaviors, a threshold or the duration of repetition as indicated in other studies. For 400 example, pacing: dog repeatedly (>3) paces around the pen in a fixed routine; wall bouncing: dog 401 repeatedly (>3) jumps up pen wall from side to side; tail-chasing: dog chases tail (> 3) for reasons 402 other than discomfort or grooming; chewing bars: dog repeatedly chews and bites at the wire of the 403 404 pen (> 20 sec) (Hetts et al., 1992; Hubrecht et al., 1992; Beerda et al., 1999; Stephen and Ledger, 2005). The complex relationship between animal welfare and repetitive behaviors suggests that 405 while these behaviors can be used as an indication of suboptimal welfare, they should never be 406 used as the only measure of welfare (Mason and Latham, 2004). 407

408

409 **5.** Conclusion

The lack of uniformity in regulatory frameworks defining minimum requirements of shelters has hindered the development of a specific tool to assess dog welfare in long-term shelters. The SQP was created responding to this need and its validity, reliability and feasibility were proven (Barnard et al., 2016). This protocol has been the first tool which can be easily applied by people from different professions (veterinarians, competent authority, shelter manager, et cetera) and it identifies critical areas requiring intervention.

The feedback obtained from its dissemination and application necessitated the improvement of the 416 tool by developing a second version of the protocol (SQP2). The changes made in the protocol did 417 not modify its applicability or the reliability of the measures included. The good level of agreement 418 obtained in this study confirmed that SOP2 remains a useful tool for welfare assessment of dogs 419 housed in long-term shelters with broad areas of application (i.e., rank dogs' rescue and commercial 420 or breeding facilities according to the level of welfare they are providing). The SOP2 showed its 421 potential in detecting the impact of seasonal conditions on animal welfare. Particularly, some 422 measures included in the SQP2 such as "Presence of diarrhea" and "Number of animals panting" 423 showed their sensitivity in assessing changes in dog welfare due to different seasonal conditions. 424 Further investigations are required to confirm the utility of "Number of animals 425 shivering/huddling". Although "Lameness", "Coughing" and "Skin condition" showed results in the 426 expected direction but not significantly. These measures also require further research, for example, 427 428 including a larger sample size to explore more extensively their sensitivity to different seasons.

429

430 **Conflict of interest**

431 The authors declare that there are no known conflicts of interest associates with this publication and432 the financial support for this project didn't influence its outcomes.

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439 Authors' contributions

440	All authors conceived and designed the study. Berteselli and Arena, refined the protocol (SQP),
441	recruited the shelters, applied the SQP2 on-field in the selected shelters, collected the data and
442	drafted the manuscript. Candeloro performed the data analysis and advised on the study design, and
443	collaborated for the interpretation of the results. Dalla Villa was the responsible of operative unit for
444	animal welfare assessment and the supervisor of on-field activities. He also revised and approved
445	the final manuscript. De Massis was the supervisor on the entire project.

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581

582 Tables

Table 1: SQP2 measures associated with welfare principles and criteria. Type of measures defined in brackets; management based measure (MBM); resources based measure (RBM); animal based measures (ABM). Measures were assessed according to different three units of assessment: the shelter (evaluate the shelter as a unit and all the animals within); the pen (evaluate the pen as a unit, taking in to account all of the dogs housed in the pen); the individual (evaluate each animal as a unit).

Principle	Welfare criteria	Welfare measure (type)	Sub-measures	Unit of assessment
		Body condition (ABM)	\mathcal{P}	Individual
Good feeding	Absence of prolonged hunger	Feeding (MBM)	Feeding regimen Type of diet Special diets	Shelter
	Absence of prolonged thirst	Water supply (RBM)	Type of drinkers Availability of water Cleanliness of water Safety of drinkers	Pen
	Comfort around resting	Bedding (RBM)	Type of bedding At least one bed/dog Safety of bedding Cleanliness of bedding	Pen
		Safety of pen (RBM)	Sharp edges	Pen
		Cleanliness of animals (ABM)		Individual
Good housing		Thermoregulation (ABM)	Signs of thermal discomfort	Individual
	Thermal comfort	Shelter from adverse weather condition (RBM)	Shelter from excessive sun; wind; rain Air circulation	Pen
	Ease of movement	Space allowance (RBM)		Pen
	Absence of injuries	Skin condition (ABM)	Presence of wounds; hair loss areas, swelling areas; evidence of parasites	Individual
		Lameness (ABM)		Individual
C I M	Absence of disease	Evidence of pain (ABM) Signs of diarrhea (ABM) Coughing (ABM)		Pen
Good health		Mortality (MBM)	Euthanasia for clinical problems; behavioral problem; Deaths (other than euthanasia)	Shelter
	Absence of pain induced by management procedures	Surgeries and control pain (MBM)	Presence of operating procedures for post-surgical monitoring; Presence of hospital pens Presence of protocol of analgesia	Shelter

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	Expression of social behaviors	Social housing (MBM)	Single housing pens Pair housing pens Group housing pens (≤ 5) Group housing pens (> 5)	Shelter		
	Expression of other behaviors	Abnormal behavior (ABM)	Repetitive and compulsive behaviors	Pen		
		Barking (ABM)		Pen		
Appropriate		Exercise (MBM)	Exercise in outdoor areas Walking at leash	Shelter		
behavior		Reaction to human (ABM)		Individual		
	Good human-animal relationship	Training and rehabilitation (MBM)	Presence of training personnel for activities with dogs, and specialized personnel in behavioral rehabilitation	Shelter		
	Positive emotional state	Emotional state (ABM)	QBA	Pen		

 Labilitation (Emotional state (ABM)

Table 2: Refinements of SQP performed to develop the SQP2

Variable	SQP	SQP2	
Nasal discharge	At individual level	Eliminated	
Dyspnea	At individual level	Eliminated	
Morbidity	At shelter level	Eliminated	
Signs of diarrhea	At individual level	At pen level	
Coughing	At individual level	At pen level	
Evidence of pain	At individual level	At pen level	
Barking level	At shelter level	At pen level	
Emotional state	At shelter level	At pen level. The adjectives' list was	
		also refined	
Number of dogs entered in shelter	-	Added	
Number of adopted dogs	-	Added	
Number of dogs returned to owner	-	Added	
Number of dogs returned after	-	Added	
adoption	<u>_</u>		
Presence of qualified personnel for	-	Added	
dog training			
Presence of qualified personnel for	-	Added	
behavioral rehabilitation			
Thermal comfort (good housing)	Indoor/outdoor area	Shelter from adverse weather	
		conditions" (sun, wind, rain)	
Defensive and offensive aggression	Considered as two separate Considered as a unique categor		
	categories		

Table 3: Sample size. The sampling includes only dogs over 6 months age and that have been in

shelter for 2 months.

Total number of housed dogs	Number of dogs to assess
Up to 29	All dogs
30-59	30
60-89	40
90-139	50
140 over	60

599 Table 4: Scoring system of quantitative and qualitative variables.

Quantitative variables	Туре	Score	Qualitative variables	Туре	Score
Aggressive	CV	VAS from 0 to 125 mm	Active-repetitive behaviours	CatV	Y-N
Alert	CV	VAS from 0 to 125 mm	Adequacy of pen area	CatV	Y-N
Anxious	CV	VAS from 0 to 125 mm	Age classes	CatV	Y-N
Barking level	CV	VAS from 0 to 125 mm	Air circulation	CatV	Y-N
Comfortable	CV	VAS from 0 to 125 mm	Body Condition	CatV	Adequate Too thin Too fat
Curious	CV	VAS from 0 to 125 mm	Clean water	CatV	Y-N
Durand	CV	VAS from 0 to 125	Cleanliness	CatV	Y-N
Depressed	CV	mm	Coughing	CatV	Y-N
Evoited	CV	VAS from 0 to 125 mm	Dry/clean bedding	CatV	Y-N
Excited			Evidence of pain	CatV	Y-N
Fearful	CV	VAS from 0 to 125 mm	Fear/aggression test	CatV	Sociable Only fear Offensive/ defensive aggression
Hesitant	CV	VAS from 0 to 125 mm	Lameness	CatV	Y-N
Playful	CV	VAS from 0 to 125 mm	One bedding/dog	CatV	Y-N
Relaxed	CV	VAS from 0 to 125 mm	Safe bedding	CatV	Y-N
Nervous	CV	VAS from 0 to 125 mm	Sharp edges	CatV	Y-N
Sociable	CV	VAS from 0 to 125 mm	Shelter from adverse weather conditions (sun, wind, rain)	CatV	Y-N
Number of animals > 20Kg	DV	Ordinal number	Signs of diarrhoea	CatV	Y-N
Number of animals \leq 20Kg	DV	Ordinal number	Skin condition	CatV	Y-N
Number of animals shivering/huddling	DV	Ordinal number	Type of bedding	CatV	Y-N
Number of animal painting	DV	Ordinal number	Type of drinkers	CatV	Y-N

600

CV: continue variable; DV: discrete variable; CatV: categorical variable; VAS: visual analogue scale; Y-N:yes-no

602 Table 5: Inter-observer agreement

	Pearson' correlation		Cohen's Kappa
Quantitative variables	Cor.P	Qualitative variables	K
Aggressive	0,72*	Active-repetitive behaviours	0,30*
Alert	0,60*	Adequacy of pen area	0,85*
Anxious	0,60*	Age classes	0,89*
Barking level	0,61*	Body Condition	0,83*
Comfortable	0,74*	Clean water	0,66*
Curious	0,74*	Cleanliness	0,70*
Depressed	0,51*	Dry/clean bedding	0,60*
Excited	0,65*	Fear/aggression test	0,83*
Fearful	0,83*	Lameness	0,82*
Hesitant	0,60*	One bedding/dog	0,65*
Playful	0,70*	Safe bedding	0,64*
Relaxed	0,74*	Sharp edges	0,72*
Nervous	0,67*	Shelter from rain	0,89*
Sociable	0,84*	Shelter from strong wind	0,88*
Number of animals > 20Kg	0,91*	Signs of diarrhoea	0,77*
Number of animals ≤ 20 Kg	0,92*	Skin condition	0,84*
Number of animals shivering/huddling	1*	Type of bedding	0,77*
		Type of drinkers	1*

⁶⁰³ *z-score, P < 0.001. Level of agreement in according to Landis and Kock (1977): 0.00, less than chance

agreement; 0.01-0.20 slight agreement; 0.21-0.40 fair agreement; 0.41-0.60 moderate agreement; 0.61-0.80

substantial agreement; 0.81-0.99 almost perfect agreement; 1 perfect agreement.

Table 6: Temperature recorded during the assessments.

Shelter region	Winter		Summer	
	Temperature	Humidity	Temperature	Humidity
Abruzzi*	9°C	74%	24°C	61%
Marche	9°C	76%	27°C	42%
Molise	10°C	70%	28°C	50%

*For Abruzzi the average of winter and summer temperatures and humidity were calculated over the three

608 shelters' assessments.

Highlights

- We refined the validation of dog welfare assessment protocol Shelter Quality (SQP)
- We assessed the Reliability of SQP measures through inter-observer agreement
- We assessed the Sensitivity of SQP animal-based measures to climatic changes
- Diarrhea and panting were sensible measures in assessing welfare between seasons
- Level of agreement on lameness, body condition, skin condition was high