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Through the Lens of Age: Using Dog Photographs to Uncover Welfare and Stress

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Abstract: This study evaluated the potential of using dogs' apparent age, judged from photographs, as a non-invasive tool for assessing their welfare. Traditional welfare assessment methods often rely on behavioral and physiological indicators, which can be resource-intensive and invasive. This research explored whether apparent age, a measure used in humans to predict health and longevity, can also serve as an indicator of welfare in dogs by investigating its association with relative telomere length (RTL), a biomarker of biological aging. Photographs of 60 domestic dogs were evaluated by canine specialists and general volunteers via the citizen science platform Zooniverse. Participants estimated the age of 20 dogs from three different age categories: young (0–2 years), adult (2–5 years), and senior (6+ years). The accuracy of these predictions was compared to the dogs' chronological ages and RTLs. Generalized linear models were used to assess factors influencing prediction accuracy, including the dogs' age, sex, and origin. Results indicated that both specialists and volunteers reliably estimated the age of senior dogs, with no significant differences in accuracy between groups. Dogs with accurate apparent age estimates had RTLs matching their chronological age, while those with premature aging signs had shorter RTLs. This suggests apparent age could be a practical, non-invasive welfare assessment tool, offering a potentially accessible method for new welfare assessment protocols.

Keywords: apparent age; telomere length; dog welfare; biological aging; citizen science; canine health assessment

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Citation: Dutra, L.M.L.; Vasconcellos, A.d.S.; Young, R.J. Through the Lens of Age: Using Dog Photographs to Uncover Welfare and Stress. *Pets* **2024**, *1*, 372–386. https://doi.org/10.3390/pets1030026

Academic Editor: Jan S. Suchodolski

Received: 30 September 2024
Revised: 4 November 2024
Accepted: 8 November 2024
Published: 15 November 2024

1. Introduction

Aging, much like growth, varies among individuals; this is why the concept of biological age, distinct from chronological age, has been widely studied [1]. Biological age, also known as 'apparent age', can be assessed through photographs by evaluating features such as skin texture, pigmentation, eye and mouth characteristics, and hair color, which are considered key indicators in humans [2]. In humans, apparent age is a robust predictor of mortality and age-related diseases, as it correlates with cortisol levels and telomere length, which are both indicators of stress [3,4]. Studies with chimpanzees (*Pan troglodytes*) also show a strong link between apparent age and health, with participants accurately assessing health from photographs [5]. Other research found that apparent age is related to bone loss in wild chimpanzees [6]. In wildlife, predation and habitat loss are significant pressures contributing to accelerated aging [7]. Dogs experience similar physical changes with age, such as muscle loss, graying hair, and the development of cataracts, which mirror human aging processes [8]. Interestingly, research has shown that young dogs suffering from anxiety may exhibit premature aging, with noticeable graying of the face [9].

Accelerated telomere attrition is linked to cardiovascular diseases, diabetes, poor diet, lack of exercise, and chronic stress [10,11]. Chronic stress raises cortisol levels, contributing to skin aging, which plays a key role in determining apparent age [3,4]. Comparing perceived age to chronological age can serve as an indicator of health, with biomarkers such as telomere length providing insights into cellular aging [3]. Despite the established connections between chronic stress, telomere length, and aging, no studies have yet explored these factors together in non-human mammals. However, these parameters are conserved across mammals [12], suggesting that similar associations could exist in dogs.

Animal welfare is increasingly recognized as a multidimensional concept, involving not just biological health but also the emotional state of the animal and its ability to express natural behaviors [13,14]. It is widely accepted that a comprehensive assessment of welfare must include these three interconnected elements: the animal's physical condition (biological functioning), its emotional experiences, and its capacity to engage in behaviors that are normal for its species [15,16]. Given this complexity, using a single tool to assess welfare, such as apparent age, could be limiting. However, apparent age—particularly when combined with behavioral evaluation—can provide valuable insights into the mental aspect of welfare, offering a non-invasive indicator of the animal's stress and overall health.

Citizen science has further expanded the scope of welfare studies by involving the public in research, allowing non-experts to provide reliable data in tasks like image classification [17]. Dogs, with their close relationships to humans and widespread presence in households—40% of U.S. households own a dog, and 9.4 million companion dogs live in the UK [18,19]—are especially well-suited for such studies, where tools like apparent age can be assessed at scale and combined with public involvement to enhance welfare evaluations.

Current methods for assessing animal welfare primarily rely on behavioral and physiological parameters, which can be both time-consuming and costly. This study investigated the potential of a new tool for welfare assessment that could be used by non-specialists. If apparent age, as determined from a photograph, proves to be an effective indicator, it could offer a more practical and affordable method for various stakeholders, including pet owners, veterinarians, kennel owners, police dog handlers and shelter managers. This approach would simplify welfare assessments, allowing for broader and more frequent evaluations of canine health and welfare. This study aimed to validate the use of apparent age as a tool for assessing animal welfare, offering a potentially accessible method for veterinarians and dog shelters to build a new welfare assessment protocol.

2. Methods

2.1. Ethics

The data collection for this study was conducted under ethical approval number STR1617-22, granted by The University of Salford's Ethics Committee. All participating owners and institutions were fully informed about the study's aims and provided with an invitation letter, detailed information, and a consent form (Appendices A–C). The collection of biological material was authorized by DEFRA under license number ITIMP16.1096.

2.2. Dog Subjects

Standardized photographs (i.e., same camera, lens, set-up, etc.) were taken of all 264 domestic dogs used in the study. These dogs represented a variety of categories: pet dogs, shelter dogs, police dogs, laboratory dogs, rehomed dogs, and dogs involved in behavioral research.

2.3. Photograph Collection and Classification

Multiple photographs were taken of each dog using a Nikon D7200 Digital SLR. The two highest-quality images of each dog facing the camera were selected. Photos were cropped to show only the head, with minimal inclusion of the neck or background. When necessary, Adobe Photoshop© CC 2015 and Adobe Lightroom© were used to blur the background and enhance focus on the dog, ensuring that extraneous elements did not interfere with the evaluation [5].

The categorization of the dogs' age was based on consultations with veterinarians regarding canine development and the prevalence of age-related diseases. We also referenced the guidelines provided by [20], to establish the following age categories: young: 0–2 years, adult: 2–5 years, senior: over 6 years. This approach ensured a comprehensive and scientifically informed classification system. For dogs aged between categories (e.g., 3 years and 3 months), their age was rounded down if less than six months, and up if more.

2.4. DNA Sampling and Telomere Length Measurement

Buccal swab samples were collected either by LMLD or the owner or keeper, or by a vet accompanied by LMLD (i.e., whoever was more appropriate for the sampling and would cause the least discomfort to the animal), and then LMLD labeled and stored the samples. The swab was placed against the inside surface of the dog's cheek, and saliva and tissue were collected by rolling the Isohelix Buccal Swab (Cell Projects, Kent, UK) against the cheek. After that, the dog was rewarded through positive reinforcement. To prevent DNA degradation, a Dri-Capsule (Cell Projects, Kent, UK) was included in each swab tube, enabling the sample to be stored at room temperature [21]. LMLD was responsible for ensuring that the samples were securely labeled and stored until further analysis.

DNA was extracted from dogs' buccal cell samples by using a Buccalyse DNA Release Kit following the manufacturer's protocol. The concentration of DNA extracted from the swab samples was determined using a NanoDrop® ND-1000 UV-Vis Spectrophotometer (Thermo Fisher Scientific, Oxford, UK).

Telomere length was measured using quantitative PCR (qPCR). Each run involved two key components: (i) a tenfold serial dilution of a DNA pool from 264 dogs (100.7 ng/µL) to generate a standard curve for primer optimization and quality assurance; and (ii) no template controls (NTC) to detect possible contamination or primer dimer formation. This was applied to both the telomere and reference genes, with each dilution and NTC run in triplicate [22]. The primers used for the telomere analysis were based on [23] design: telg (5′-ACACTAAGGTTTGGGTTTGGGTTTGGGTTTGGGTTAGTGT-3′) and telc (5′-TGTTAGGTATCCCTATCCCTATCCCTATCCCTATCCCTAACA-3′). For the reference gene, 18S primers were utilized, with the sense primer (5′-GAGGTGAAATTCTTGGACCGG-3′) and antisense primer (5′-CGAACCTCCGACTTTCGTTCT-3′) derived from [24]. Primer performance for each reaction was calculated using Rotor-Gene Q software (version 2.3.1), with efficiencies ranging between 98% and 100%. We adapted the monochrome multiplex qPCR assay from [22] for this study, following the master mix preparation protocol outlined by [25].

Amplifications were conducted using the Rotor-Gene Q cycler (QIAGEN) with the corresponding 0.1 mL strip tubes and caps provided by the manufacturer. The reactions were managed via the Rotor-Gene Q software (version 2.3.1), which also generated a standard curve. This curve corresponded to the dilution factors of the standards for telomere and reference gene measurements for each sample. The qPCR reaction mix and conditions followed those previously described in [26].

The relative telomere length (RTL) was determined using a modified version of [27] qPCR method, where a multi-copy gene was used as the reference, as validated by [28]. The telomere-to-single-copy gene (T/S) ratio was calculated by comparing the telomere repeat copy number to the reference gene copy number. Each sample was assayed in duplicate, and if the results varied, a second assay was conducted. The final RTL for each sample was the average of the duplicate measurements [27].

2.5. Evaluation of Dog Photographs by Specialists

In this study, specialists were defined as participants with formal training or professional experience in dog care, such as veterinarians or dog trainers, who were invited to evaluate the photographs. Information on their sex, age, job title, and experience with dogs was collected. Specialists were recruited through word of mouth, with the questionnaire initially shared with a dog trainer, who then distributed it within her network. Each participant received a link to access an online questionnaire via Google Forms, featuring 60 dog photographs, 20 from each age category, to be classified as "young", "adult", or "senior". The 60 photographs were chosen randomly among the 262 photographs. In total, 53 assessors completed the evaluation, providing anonymous ratings without any additional information about individual dogs. In addition to the photograph-based age evaluations, the survey included supplementary questions to gather more detailed information from participants. These questions focused on their perceptions of the dogs' health and welfare and their previous experience with dogs (e.g., pet owners, veterinarians, or trainers).

2.6. Evaluation of Dog Photographs by Non-Specialist General Public Volunteers

To compare the specialists' evaluations with non-specialists, we invited general public members with no formal dog training through the Zooniverse platform. Each non-specialist was provided with written instructions outlining how to assess the apparent age of the dogs based on visual cues such as fur condition, facial features, and overall body posture (see the non-specialist instructions in Supplementary Materials Figures S1–S8). This ensured a standardized approach across all evaluations and provided additional context for understanding how participants approached age estimation. The same 60 dog photographs assessed by specialists were uploaded to the platform and shown to volunteers for anonymous classification. No demographic data regarding non-specialists' age, sex, or dog-related experience was collected. Non-specialists were also asked to classify dogs into the same three age groups (0–2 years, 2–5 years, and over 6 years). Each image was evaluated by 33 volunteers, and detailed instructions, including a video tutorial and help tab, were provided to guide the volunteers in their task.

2.7. Data Analysis

The initial analysis calculated the percentage of correct age category predictions from the dogs' photographs, based on both specialist and non-specialist evaluations. From this, we derived the probability of a dog's age being correctly assessed. A discriminant analysis was performed to determine whether age grouping (i.e., young, adult, or senior) could be accurately predicted based on these evaluations. Next, we selected the top 10 dogs with the most accurate and the 10 with the least accurate age predictions from both specialists and non-specialists for further analysis.

The distribution of the data was assessed using the Shapiro–Wilk test. The results indicated that the data were normally distributed, which guided the selection of subsequent statistical analyses.

We used a generalized linear model (GLM) to examine whether factors such as the dog's age, sex, and origin (shelter, pet, or work) influenced the accuracy of age predictions by both specialists and non-specialists. Another GLM was applied to assess the impact of the specialists' sex, age, job, and experience on their success in predicting dog ages. Variables that were not significant were removed from the model using the drop1 function, and model selection was guided by Akaike's Information Criterion (AIC).

Both sets of responses—specialists and non-specialists—were analyzed separately before being combined to evaluate the factors that helped or hindered accurate age predictions. Normality was tested using the Kolmogorov–Smirnov test, and statistical significance was set at $p < 0.05$. For this analysis, 60 dogs (20 young, 20 adults, and 20 seniors) were included. Given that there were three categories to choose from, random guesses would yield a 33.3% accuracy rate.

All statistical analyses were performed using RStudio (RStudio Team, 2016) and Minitab 18 (Minitab Inc., State College, PA, USA, 2010). LMLD conducted on all of the sample analyses, including DNA extraction, qPCR, and data evaluation. Photographs were taken to document the process and results.

3. Results

3.1. Volunteer Recruitment Process

Zooniverse volunteers were recruited through the platform itself, where research invitations are advertised, allowing participants to voluntarily opt into the study.

3.2. Participants

Statistical analysis comparing the two groups of assessors revealed no significant difference in their ability to estimate dog age from photographs. This finding suggests that non-specialists, aided by visual cues and citizen science tools, can perform similarly to specialists in this task. These results highlight the potential for broad application of the apparent age tool in welfare assessments.

3.3. Accuracy of Age Predictions

By combining the assessments from both specialists and Zooniverse non-specialists, we calculated an average score for the correct predictions of each dog's age. Dogs with a correct prediction rate of 28% or less were deemed statistically significant for being incorrectly assessed, while those with a rate of 38% or more were significantly correctly-assessed.

3.4. Specialist Predictions

The photographs were evaluated by 53 specialists, of whom 43 were women and 10 men. The age range of the specialists was predominantly between 25 and 39 years. Most of the assessors were dog trainers (71.7%), with others identified as biologists (3.77%), researchers (15.09%), veterinarians (7.55%), and a biology student (1.89%).

Specialists correctly predicted the ages of the dogs with an overall accuracy of 56.7% (Table 1). The top 10 most accurately predicted dogs included seven senior dogs, two adults, and one young dog (see 9 of the photographs in Supplementary Materials Figures S9–S17). Correct predictions ranged from 69.8% to 98.1% for these top-performing individuals (Table 2). Conversely, the least accurate predictions included five senior and five young dogs, with correct guesses as low as 0.00% (see 9 of the photographs in Supplementary Materials Figures S18–S26) (Table 3).

Table 1. Discriminant analysis for specialists' correct answers for dogs' apparent age prediction from photographs from dogs' photographs, as a function of their true age category.

	True Age Category Group		
Put into Group	**Adult**	**Senior**	**Young**
Adult	10	6	6
Senior	7	12	2
Young	3	2	12
Total N	20	20	20
N correct	10	12	12
Proportion	0.500	0.600	0.600

Table 2. Dogs that had their ages most accurately estimated (%) by dog specialists, from photographs.

Dog ID	Age	Sex	Category	Correct Predictions (%)
FPS101_F_7	Senior	Female	Pet	69.81
FPS28_F_3	Adult	Female	Pet	71.70
FPS48_M_4	Adult	Male	Pet	73.58
FPS15_F_1.33	Young	Female	Pet	79.25
FPS73_M_7	Senior	Male	Pet	81.13
MPS11_M_12	Senior	Male	Work	81.13
PS18_F_13	Senior	Female	Pet	81.13
FPS39_M_10	Senior	Male	Pet	96.23
PBS5_F_6	Senior	Female	Work	98.11
SS17_M_10	Senior	Male	Shelter	98.11

Table 3. Dogs that had their ages least accurately estimated (%) by dog specialists, from photographs.

Dog ID	Age	Sex	Category	Correct Predictions
SS45_M_2	Young	Male	Shelter	0.00
FPS60_F1.5	Young	Female	Pet	3.77
FPS117_F_9.5	Senior	Female	Pet	5.66
MPS12_M_12	Senior	Male	Work	9.43
FPS89_M_1	Young	Male	Pet	13.21
PS1_M_7	Senior	Male	Pet	16.98
PS16_M_8	Senior	Male	Pet	16.98
WKDS12_F_5	Senior	Female	Rehome	18.87
FPS31_M_0.41	Young	Male	Pet	20.75
PBS10_M_2	Young	Male	Work	20.75

A generalized linear model revealed that the specialists' success in predicting dog ages was significantly influenced by their level of experience, with professionals having more than 15 years of experience outperforming those with fewer years of experience ($r^2 = 0.190$, $p < 0.05$; Table 4, Figure 1).

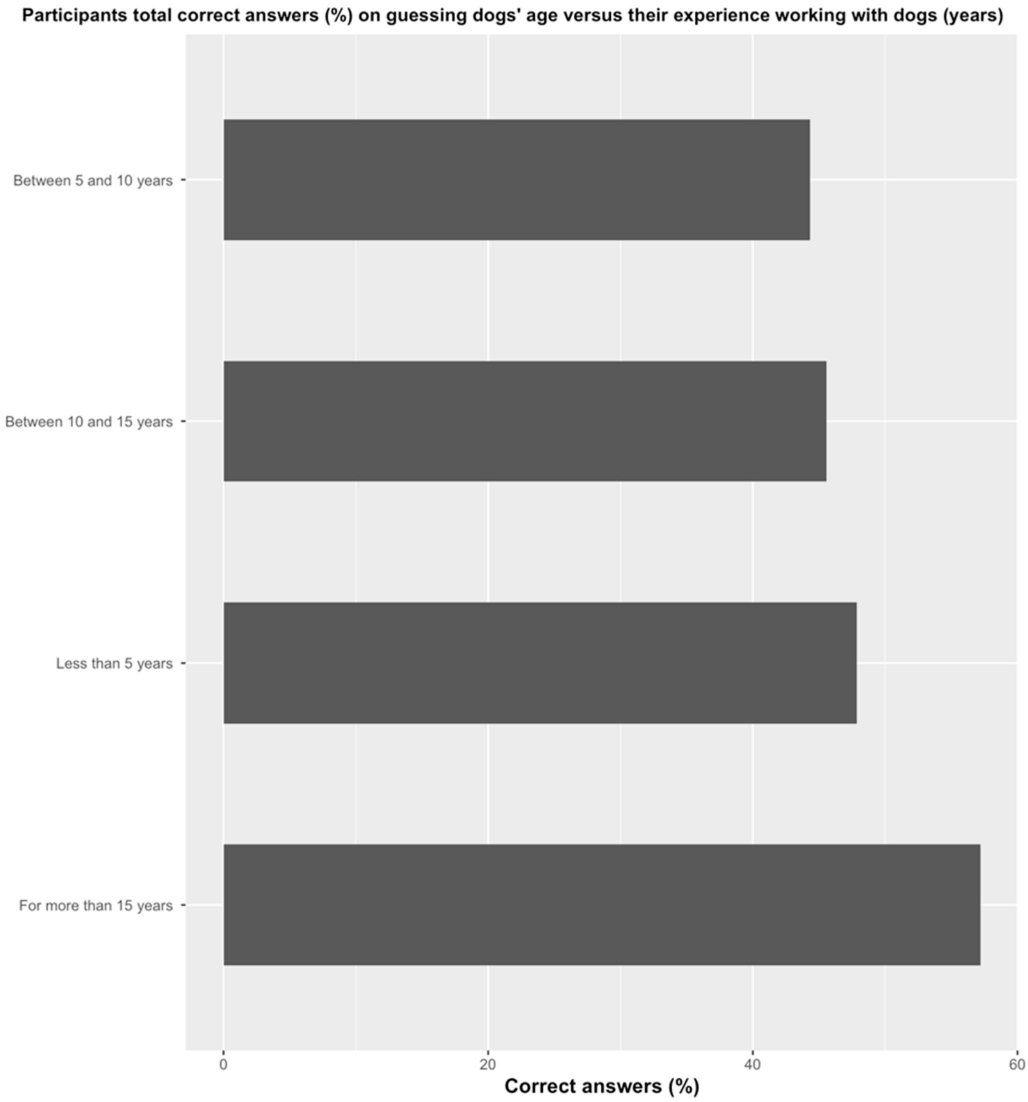

Figure 1. Dog specialist correct dogs' age predictions (%) based on the specialist's time of experience with dogs (from photographic data).

Table 4. Final generalized linear model result for the effects of specialists' time of experience with dogs on dogs' apparent age correct prediction by them.

Parameters [ab]	**Estimate ± SD**	**t Value**	***p***
Intercept	45.557 ± 2.647	17.20	<0.0001 ***
Between 5 and 10 years	−1.223 ± 3.018	−0.405	0.6872
For more than 15 years	11.667 ± 4.585	2.544	0.0142 *
Less than 5 years	2.291 ± 2.960	0.744	0.4426

Non-significant effects of parameters and interactions not shown were removed during the model selection process using the function drop1. Best models for: dogs' apparent age prediction by dog specialists AICc = 355.68. Explanatory variables included in the full model: specialist's sex, age, job and experience time with dogs. Less than 5 years; Between 5 and 10 years; Between 10 and 15 years; For more than 15 years: Between 10 and 15 years. * $p \leq 0.01$, *** $p \leq 0.0001$.

3.5. Volunteer Predictions

Similarly, the same dogs were evaluated by 33 non-specialists through the Zooniverse platform. The non-specialists overall accuracy in predicting dog ages was 51.7% (Table 5). As with the specialists, the top 10 most accurately predicted dogs included seven senior dogs, two young dogs, and one adult. Correct predictions for these individuals ranged from 63.64% to 96.97% (Table 6).

Table 5. Discriminant analysis for non-specialists' correct answers for dogs' age prediction by dogs' age categories (from photographic data).

		True Group	
Put into Group	**Adult**	**Senior**	**Young**
Adult	5	3	4
Senior	9	13	3
Young	6	4	13
Total N	20	20	20
N correct	5	13	13
Proportion	0.250	0.650	0.650

Table 6. Dogs that had their ages most accurately estimated from photographs by Zooniverse non-specialists.

Dog ID	**Age**	**Sex**	**Category**	**Correct Predictions (%)**
PS18_F_13.jpg	Senior	Female	Pet	63.64
FPS4_F_9.jpg	Senior	Female	Pet	66.67
WKDS9_F_2.jpg	Young	Female	Rehome	66.67
FPS68_F_3.jpg	Adult	Female	Pet	69.70
FPS15_F_1.33.jpg	Young	Female	Pet	69.70
FPS73_M_7.jpg	Senior	Male	Pet	78.79
FPS39_M_10.jpg	Senior	Male	Pet	81.82
MPS11_M_12.jpg	Senior	Male	Work	81.82
PBS5_F_6.jpg	Senior	Female	Work	90.91
SS17_M_10.jpg	Senior	Male	Shelter	96.97

The least accurate predictions from non-specialists mirrored the specialists' findings, with both groups incorrectly assessing several senior and young dogs (Table 7).

Table 7. Dogs that had their ages least accurately estimated from photographs by non-specialists.

Dog ID	**Age**	**Sex**	**Category**	**Correct Predictions (%)**
FPS26-F_1.5.jpg	Young	Female	Pet	0.00
FPS31_M_0.41.jpg	Young	Male	Pet	3.03
FPS60_F_1.5.jpg	Young	Female	Pet	3.03
SS45_M_2.jpg	Young	Male	Shelter	3.03
PS1_M_7.jpg	Senior	Male	Pet	9.09
FPS117_F_9.5.jpg	Senior	Female	Pet	12.12
MPS12_M_12.jpg	Senior	Male	Work	12.12

Table 7. *Cont.*

Dog ID	Age	Sex	Category	Correct Predictions (%)
PS16_M_8.jpg	Senior	Male	Pet	15.15
FPS113_M_7.jpg	Senior	Male	Pet	18.18
WKDS12_F_5.jpg	Senior	Female	Rehome	18.18

3.6. Comparison Between Specialists and Non-Specialists

A *t*-test revealed no significant difference between the accuracy of specialists (mean = 46.8, SD = 22.8) and non-specialists (mean = 41.5, SD = 21.9) in predicting dog ages ($t(117) = 1.30$, $p > 0.05$). Interestingly, seven dogs appeared in the top 10 most accurately predicted by both groups, indicating consistency in age assessment (Tables 2 and 6). Eight dogs also appeared in the top 10 least accurately predicted, underscoring shared challenges in identifying certain age groups.

3.7. Factors Influencing Age Prediction

When asked what features helped them predict the dogs' ages, 87% of participants cited hair color as the most important factor, followed by the dogs' facial expressions (61%). On the other hand, 32% of specialists found hair type (e.g., short or long) to complicate their predictions, and 24% identified hair color as a challenge. Participants reported that the most commonly used features for judging a dog's apparent age were coat condition (cited by 72%), eye brightness (65%), and posture (58%). Those with prior professional experience, such as veterinarians and trainers, were more likely to emphasize posture and muscle tone, while non-specialists tended to focus on coat and facial appearance. These supplementary insights support the idea that a variety of visual cues contributed to the accuracy of age estimation, complementing the primary photograph-based evaluations (Figures 2 and 3).

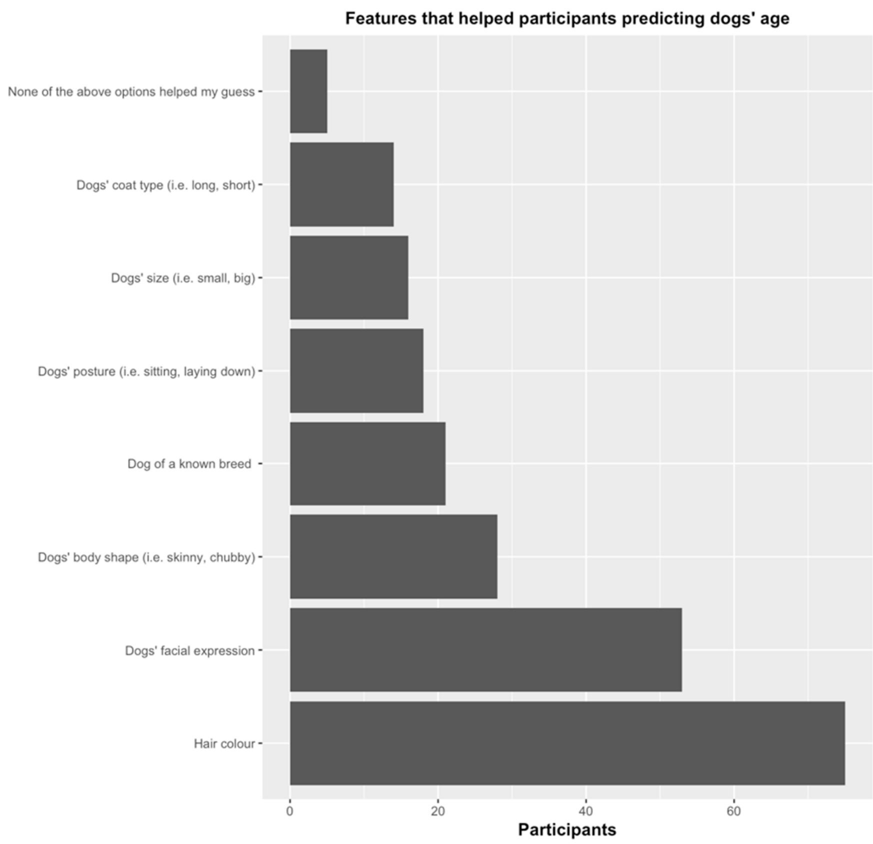

Figure 2. Features that helped participants make predictions of dogs' ages from photographs.

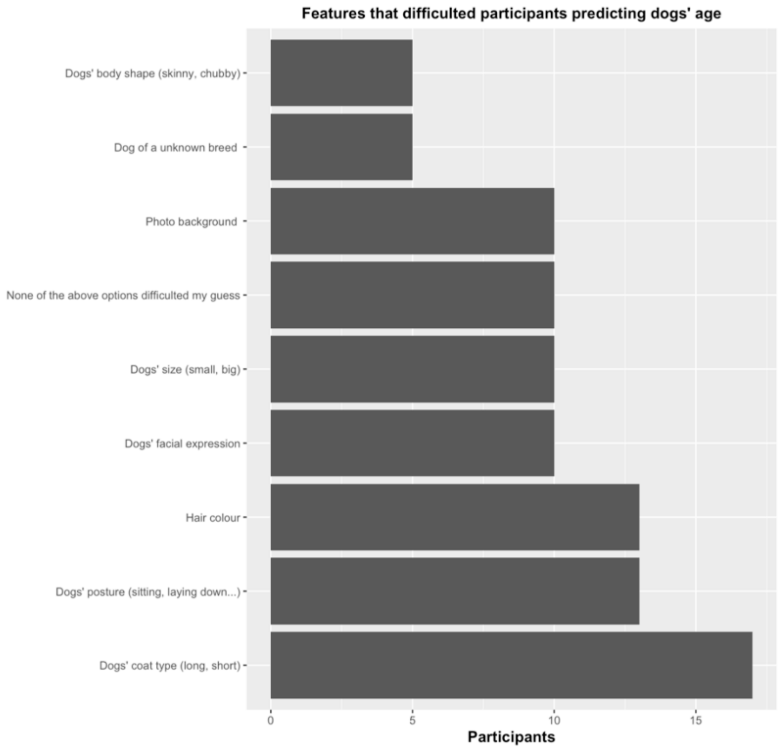

Figure 3. Features that hindered participants' predictions of dogs' ages from photographs.

3.8. Relationship Between Apparent Age and Relative Telomere Length

When comparing apparent age predictions with the dogs' relative telomere lengths (RTL), we found that five out of the seven senior dogs with accurate age predictions had RTLs within the expected range for their age (Table 8, Supplementary Materials Figures S4–S13). Among the young dogs, one had a longer RTL than expected for her age, while another had an RTL within the expected range.

Table 8. Dogs that had their age most accurately predicted from photographs by specialists and non-specialists.

Dog ID	Age	Sex	Category	Indiv. rTL	rTL Expected for Age	CI (95%)
1	Senior	Male	Pet	0.747	0.742	(0.658, 0.829)
2	Senior	Male	Work	0.570	0.674	(0.442, 0.904)
3	Senior	Female	Work	0.820	0.751	(0.710, 0.774)
4	Senior	Female	Pet	0.646	0.792	(0.660, 0.939)
5	Senior	Male	Shelter	0.792	0.745	(0.714, 0.787)
6	Senior	Female	Pet	0.724	0.752	(0.710, 0.774)
7	Senior	Female	Pet	0.736	0.742	(0.729, 0.770)
8	Young	Female	Pet	0.744	0.750	(0.722, 0.771)
9	Young	Female	Rehome	0.846	0.750	(0.729, 0.770)

rTL = relative Telomere Length.

Conversely, dogs whose ages were least accurately predicted often had RTLs outside the predicted range for their chronological age. Several young dogs with prematurely graying hair had shorter RTLs, a marker of accelerated biological aging (Table 9, Figures S19–S26).

Table 9. Dogs that had their age least accurately predicted from photographs by specialists and non-specialists.

Dog ID	Age	Sex	Category	Predicted	Indiv. rTL	rTL Expected for Age	CI (95%)
10	Senior	Female	Pet	Adult	0.683	0.745	(0.710, 0.774)
11	Senior	Male	Work	Young	0.751	0.674	(0.442, 0.904)
12	Senior	Male	Pet	Adult	0.776	0.742	(0.714, 0.787)
13	Senior	Male	Pet	Adult	0.902	0.801	(−1.047, 2.637)
14	Senior	Female	Rehome	Adult	0.811	0.751	(0.658, 0.829)
15	Senior	Male	Pet	Young	0.670	0.742	(0.695, 0.804)
16	Young	Male	Work	Adult	0.706	0.750	(0.695, 0.804)
17	Young	Male	Pet	Adult	0.640	0.746	(0.729, 0.770)
18	Young	Male	Shelter	Senior	0.712	0.750	(0.729, 0.770)

rTL = relative Telomere Length.

4. Discussion

This study provides the first evidence linking apparent age and relative telomere length (RTL) in dogs as an indicator of animal welfare. The findings demonstrate that both specialists and non-specialists can reliably estimate a dog's age from photographs, particularly senior dogs showing visible signs of aging such as graying hair and cataracts [9,29].

Apparent age is a promising measure of biological age, as individuals who appear older than their chronological age often develop age-related diseases earlier [30]. This study shows that people, including non-specialists, can distinguish between dogs aging prematurely and those aging healthily. In cases where young dogs exhibited premature graying, the presence of stress or anxiety may have contributed to their accelerated biological aging [9].

The apparent age tool offers significant advantages as a non-invasive method to assess the biological functioning of dogs. It can be integrated into a broader welfare assessment framework to provide a comprehensive understanding of the animal's welfare. Welfare assessment must account for not only biological functioning but also the emotional and behavioral states of the animal, which are often interconnected with physical health [14]. For instance, environmental stressors in shelters can significantly impact a dog's emotional well-being, leading to premature aging [31]. By incorporating apparent age into a welfare protocol that includes behavioral observations and/or physiological markers, shelters and other institutions could gain a more holistic view of an animal's welfare and identify those in need of urgent intervention.

Interestingly, there was no significant difference in the accuracy of age predictions between specialists and non-specialists, suggesting that the close evolutionary relationship between humans and dogs enables even non-experts to assess canine age through visual cues [32]. However, due to limitations in the demographic data collected from Zooniverse participants, it is possible that some non-specialists had considerable experience with dogs, which may have influenced the results.

The ability to visually assess a dog's apparent age could have practical implications for animal welfare assessments. Dogs that are biologically aging more rapidly than their chronological age may be at increased risk of disease and reduced longevity [33]. Identifying these individuals through photographs could lead to earlier interventions, such as improved diet, exercise routines, and social enrichment, which can slow biological aging, as suggested in previous research.

One of the significant benefits of the apparent age tool is its versatility in various settings, especially in environments like shelters where dogs are often subjected to high levels of stress and suboptimal living conditions. Research has shown that environmental stressors in shelters, such as confinement, lack of social interaction, and poor stimulli, can accelerate aging and negatively impact both physical and mental health [31]. By utilizing the apparent age tool, shelter staff could quickly and non-invasively assess the biological aging of dogs. This would help identify dogs whose biological age appears advanced relative to their chronological age, signaling potential health issues or high-stress levels. Such dogs could be prioritized for medical intervention, behavioral rehabilitation, or faster rehoming, improving overall welfare.

Beyond shelters, this tool could have broad applications in other contexts. For instance, in veterinary clinics or rescue organizations, where frequent and efficient welfare monitoring is required, the tool could be an early detection system for stress-related aging or health deterioration. Its non-invasive nature makes it suitable for routine welfare assessments in settings with limited resources or high animal turnover, such as dog daycare facilities. Additionally, this tool could complement existing welfare protocols, providing a more comprehensive understanding of an animal's well-being when used alongside behavior and health assessments [34,35]. Future studies should explore its integration into these broader welfare frameworks, especially in environments where external stressors may play a critical role in shaping health and welfare outcomes.

This is the first study to link apparent age, telomere length, and welfare status in dogs. Although the sample size was relatively small, the findings support the use of apparent age as a possible integrative tool for welfare assessment. Future studies should explore the potential of this method in different species and environments, such as zoos or shelters, to further validate its effectiveness in assessing animal welfare. Further validation of the apparent age tool across different dog populations, breeds, and environmental conditions is recommended. This would help refine its accuracy and broaden its applicability. Additionally, it would be valuable to investigate the tool's use in long-term welfare monitoring, particularly in high-stress environments like shelters, to determine its effectiveness in identifying animals at risk of accelerated aging due to chronic stress.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pets1030026/s1.

Author Contributions: Conceptualization, R.J.Y. and L.M.L.D.; methodology, R.J.Y. and L.M.L.D.; software, L.M.L.D.; validation, L.M.L.D.; formal analysis, L.M.L.D.; investigation, L.M.L.D.; resources, R.J.Y. and L.M.L.D.; data curation, L.M.L.D.; writing—original draft preparation, L.M.L.D.; writing—review and editing, L.M.L.D., A.d.S.V. and R.J.Y.; visualization, L.M.L.D., A.d.S.V. and R.J.Y.; supervision, A.d.S.V. and R.J.Y.; project administration, R.J.Y. and L.M.L.D.; funding acquisition, L.M.L.D. and A.d.S.V. All authors have read and agreed to the published version of the manuscript.

Funding: A.S.V. was funded by The Minas Gerais Research Foundation (FAPEMIG) grant number [309124/2022-0] L.M.L.D was funded by [Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)] grant number [206418/2014-0].

Institutional Review Board Statement: The animal study protocol was approved by the Ethics Committee of University of Salford (protocol code STR1617-22, 25 January 2017).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Dataset available on request from the authors.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

My name is Luisa Dutra and I am a PhD researcher at the University of Salford and I would like to invite you and your dog to participate in my research project.

Animal welfare is assumed to be influenced by the cumulative effects of the positive and negative events experienced by an individual. Measuring stress is the main mechanism to evaluate welfare. Nowadays the methods used to assess stress are not available to everyone or are invasive; with that in mind, a good measure of cumulative experience needs to be validated for animals. We believe that the way a dog looks can be related to his/her welfare and health. We aim to investigate if the used methods to assess stress are related to a dog's perceived age.

This research poses several questions. It will:

1. Test the different methods to assess dog stress.
2. Investigate how a dog's perceived age can be a predictor of their welfare.
3. Look at how changes in a dog's routine, such as play and walks, can reverse the effects of negative experiences.

I will be more than happy to provide you with more information regarding each question. You are encouraged to take part in all.

If you have any questions or require more information please do not hesitate to contact me.

Yours faithfully,

Luisa Dutra

PhD student

School of Environment and Life Sciences

University of Salford

Appendix B

Validating perceived age as a tool to evaluate canid wellbeing

I would like to invite your dog to take part in a research study. Before you decide you need to understand why the research is being done and what it would involve for your dog and yourself. Please take time to read the following information carefully.

The aim of this project is to develop a simple and non-invasive system to evaluate the cumulative lifetime experience to validate age as a reliable tool to assess dog welfare.

Nowadays, animal welfare is a widespread concern and assessing individuals' wellbeing is extensively discussed. Progress in improving animal welfare is currently limited by the lack of objective methods for assessing lifetime experience. Animal welfare is assumed to be influenced by the cumulative effects of the positive and negative events experienced by an individual. Measuring stress is the main mechanism used to evaluate welfare. One way to assess animals' life quality is measuring stress hormone levels because they are strongly associated with individual's health. Stressed individuals are more prone to develop cardiovascular diseases and have poorer immune responses. A molecular way to estimate life quality is measuring pieces of the animal's chromosomes called telomeres; once they reach a short length, they induce cell death, and thus, their length is associated with stress and life span. Looking older than your real (chronological) age is an indicator of a stressful life as you are aging biologically faster than you should be. In humans, looking old for your age, which can be assessed using facial photographs (perceived age) is associated with illness and death. A good measure of cumulative experience needs to be validated for non-human animals. The present study aims to investigate stress hormone levels, telomere lengths and the association of these factors with a dog's perceived age from a photograph.

Taking part in the research is entirely voluntary. Prior to starting the trials, I will describe the study to you. You will then be asked to sign a consent form to show you agree with your dog (and yourself) taking part. You are free to withdraw your dog at any time, without giving a reason.

The study will:

1. I will take a swab sample from your dog's cheek to check their stress level.
2. I will take a picture of your dog's face and body to evaluate their perceived age.

The only disadvantage is that some dogs don't like to have their mouth touched. To help with this issue we offer a positive training to desensitize your dog. Having a dog that is used to have its mouth touched facilitates owners to check dental health and administer medicines. All of the methods used in this study have been designed to minimize discomfort and be time effective and convenient for you and your dog. There are no risks associated with taking part in the study.

Your participation in this study will help us better understand new ways to measure stress in dogs.

All information that is collected about you during the course of the research will be kept strictly confidential. No names or personally identifiable information will be included in the thesis or any published results. The researcher will be the only person who has access to any identifiable data. Any paperwork that has been obtained, which contains any information about you, and all collected data will be securely filed in a lockable cabinet in the researcher's office and accessed only by the researcher. All electronic data will be protected by a password known only by the researcher. All data that has been collected shall be destroyed three years after the conclusion of the research.

If you withdraw your dog from the study we will destroy all of your identifiable information and video recorded observations and/or trials, but we may use the data collected up to your dog's withdrawal. Your dog will also be made anonymous in the thesis or any published results.

The results of the study will form part of the researcher's PhD thesis. The results will also be presented at conferences and may be published.

If you have a concern about any aspect of this study, you should ask to speak to the researcher, who will do their best to answer your questions (contact details can be found below).

If you remain unhappy after having spoken to the researcher, you should contact the researcher's supervisor:

If you remain unhappy and wish to complain formally, you can do this through the formal complaints procedure of the University of Salford. The student's supervisor can provide details of the complaints procedure if required.

Specific information about this research project can be obtained at any time from the researcher:

l.m.l.dutra@edu.salford.ac.uk

Appendix C

I confirm that I have read and understood the information sheet for the above study (version 1: 01/02/16) and understand what my dog's, and my, contribution will be	Yes	No
I have been given the opportunity to ask questions (face to face, via telephone and/or e-mail)	Yes	No
I agree to have my dog photographed during the study.	Yes	No
I agree to have my dog's mouth swab sampled during the study.	Yes	No
I understand that my dog's, and my, participation is voluntary and that I can withdraw my dog from the research at any time without giving any reason.	Yes	No

I understand how the researcher will use my dog's samples, who will see them, and how the data will be stored.	Yes	No

Name of participant...

Signature...

Date...

Name of researcher taking consent................................

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