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Research Article

Total Mouth Periodontitis Score as a Research Tool to Quantify Oral Health in Yucatan Minipigs

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ABSTRACT

Introduction: Minipigs are a popular model for many organs in the translational research ecosystem due to their similarities to humans. Minipigs develop naturally occurring periodontal disease, making them a compelling model in orofacial studies. However, periodontal disease influences the inflammatory status, the oral and gastrointestinal microbiome, and could impact the results of studies in porcine models. The present study aimed to adapt the canine total mouth periodontitis scoring (TMPS) system for use in Yucatan Minipigs and to compare the TMPS calculated from a full mouth evaluation to a more efficient TMPS QUICKSCORE.
Materials and methods: Computed tomographic images of the skull were obtained from twenty miniature Yucatan pigs (2-13 years, 65-92kg). All animals underwent an oral exam to assess gingival bleeding and periodontal pocket depth. Tooth root area measurements were obtained from CT images of two young, healthy miniature pigs and were used to calculate a weighting factor for each location; buccal and palatal/lingual for incisors and canines, and mesial buccal, distal buccal, mesial palatal/lingual, and distal palatal/lingual for premolars and molars. Weighting factors were used to calculate the contribution of gingivitis and periodontitis to the overall burden of periodontal disease in the mouth (TMPS FULLSCORE). An abbreviated score utilizing a subset of locations was calculated (TMPS QUICKSCORE). Lin's concordance correlation coefficient was used to assess concordance between FULLSCORE and QUICKSCORE. Spearman's correlation was used to compare scores with age and clinical parameters.
Results: Clinically younger animals had less severe disease compared to older animals, although gingival bleeding was present in all animals. The age of the animal was correlated with periodontal pocket depth but not with the degree of gingival bleeding. The extent of periodontal disease was positively correlated with periodontal pocket depth and gingival bleeding. High concordance between TMPS FULLSCORE and QUICKSCORE was found.
Conclusion: The current TMPS scoring system provided a fast and convenient way to quantify and monitor periodontal disease in Yucatan Minipigs. The TMPS is a helpful metric to describe the level of disease in animals, as advanced periodontal disease has downstream effects that alter findings in many body systems.

1. Introduction

Periodontal disease presents a serious public health threat, with one out of every two American adults aged 30 and over being affected[1]. As more culture-independent techniques for the classification of microbial communities become commonplace, it is becoming evident that the oral microbiome plays an important role not only in oral diseases such as periodontitis but also in a wide range of systemic disease processes[2]. The necessity of large animal models in research is being increasingly recognized as the limitations of translating rodent-based research to humans become

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clearer[3-5]. Similarities between the anatomy and physiology of pigs and humans result in high-fidelity models, and since the 1940s, miniature pigs have been used extensively for translational studies[6]. Naturally occurring periodontitis was observed in both male and female minipigs as early as six months of age, characterized by the accumulation of plaque and calculus, reddened and swollen gingiva, bleeding on probing, and an increased depth of periodontal pockets[6]. This makes miniature pigs an excellent model organism for studying periodontal disease; however, it is important to note that when using minipigs as a model for pathology in other body systems, periodontal disease can result in impaired barrier function and inflammation[7,8]. This chronic inflammation caused by periodontal disease has been linked to cardiovascular disease, gastrointestinal disease, many neoplastic processes, rheumatoid arthritis, and Alzheimer's disease, among others[9]. Pre-existing periodontal disease in a model animal system could impact the results of a specific study, and more information on the changes in the periodontal tissues of minipigs is required.

In clinical practice, periodontal disease is quantified by measuring periodontal pocket depth in millimeters using a calibrated dental probe, as well as assessing gingival inflammation and bleeding upon probing[10]. To quantify the disease, measurements are made at specific locations for each tooth, and the severity of the disease can vary greatly by location[11]. Therefore, in canine patients, often a mean mouth score is used to quantify the entire burden of periodontal disease in an individual[12]. However, simply using an average score per tooth or location does not account for differences in the anatomical size of the locations being evaluated[12]. Therefore, a canine total mouth periodontal score (TMPS) has been developed, which considers the differences in size between teeth and employs weighting factors to determine the contribution of periodontal disease parameters that each tooth contributes to the overall burden of disease in the oral cavity[12,13]. The present study aimed to develop a mouth scoring system that could be used as a rapid method for disease monitoring to assist in the recognition of the effect of oral health on all porcine studies and track dental disease over time by adapting the canine TMPS in the Yucatan Minipig.

2. Materials and Methods

2.1. Ethical approval

The Institutional Animal Care and Use Committee of the University of Pennsylvania, USA, approved all procedures with the protocol number 804541.

2.2. Animals

A total of twenty Yucatan miniature pigs (Sinclair Bioresources, Auxvasse, MO, USA), 11 females and nine castrated males, aged from 2 to 13 years old, weighing 65-92kg, were included in the present study. The facility consisted of an AAALAC-approved housing unit with controlled access and biosecurity precautions, with a 12-hour light-dark cycle and access to natural lighting. Animals were housed in individual pens on sterilized shredded paper bedding, allowing visual and olfactory contact with herd mates. Environmental enrichment consisted of rubber toys and scheduled human interactions with laboratory staff. All animals were subjected to a thorough clinical examination by a qualified veterinarian to assess their physical score before the study. The animals were skeletally mature and had a set of permanent adult teeth. All twenty animals underwent a head computed tomography (CT) and a complete oral exam under general anesthesia as outlined by Hoareau et al.[14] and Lehmann et al.[15].

2.3. Imaging

For CT, animals were fasted for 12 hours before induction of general anesthesia for head CT and oral exam. Each animal was induced for general anesthesia with 0.15-0.3 mg/kg intramuscular injection of midazolam (Hikma Pharmaceuticals, USA), 0.15-0.3 mg/kg of butorphanol (Zoetis, USA), and 0.02-0.04 mg/kg of dexmedetomidine (Zoetis, USA). An orotracheal tube was placed, and anesthesia was maintained with a 1.25-5% mixture of isoflurane (Dechra, UK) in oxygen via a semi-closed-circle system. An intravenous catheter was placed into the auricular vein, and isotonic fluids were administered at a maintenance dose of 2 mL/kg/hr throughout the procedure. An arterial catheter was placed in the auricular artery to monitor blood pressure, and vital signs were continuously monitored using an EKG, capnography, and pulse oximetry. Once under general anesthesia, animals were placed in sternal recumbency, and a CT exam of the head was acquired using a CereTom® portable CT (Neurologica, Corporation, MD, USA).

2.4. Total mouth periodontitis score

After completion of the CT exam, an oral speculum was placed, and the periodontal pocket depth and gingival bleeding indices were measured at predefined locations throughout the oral cavity. Periodontal pocket depth was measured using a calibrated dental probe as described by Harvey et al.[12]. Measurements were made at specific, pre-defined locations (LOC) on the buccal and palatal/lingual aspects of each incisor and canine tooth, yielding two measurements per tooth. The premolar and molar measurements were taken from four locations per tooth, including mesial buccal, distal buccal, mesial palatal/lingual, and distal palatal/lingual. In each area, the probe was placed into the periodontal pocket between the tooth and the gingiva and gently walked along the pocket until the deepest part of the pocket was reached. The depth in millimeters was recorded. A score of 0 to 3 was given for gingival bleeding upon probing (0: No bleeding, 1: Mild, 2: Moderate bleeding upon probing, and 3: Spontaneous gingival bleeding). Missing teeth and any other abnormalities were documented, and a subjective score modified from the Miller Classification system was assigned for the degree of gingival recession present (0: none, 1: mild, 2: moderate, and 3: severe)[16]. After the oral exam, 0.01-0.02 mg/kg of flumazenil (Baxter, USA) IV/IM and 0.1 mg/kg of atipamezole (Zoetis, USA) IV/IM were given as reversal agents to improve recovery.

2.5. Development of weighting factors

The skull CTs from two female animals (Table 1), aged 2 (pig 6) and 3 (pig 7) years old, with the most erupted teeth and lowest TMPS scores, were chosen to serve as the disease-free standard to develop the weighting scheme. Tooth root area measurements derived from head CTs were used to calculate the contribution of periodontal pathology at each LOC to the TMPS. The CT images from both animals were reconstructed on the CereTom® in a bone window with a 1.25mm slice thickness, then transferred to a specialized medical imaging viewer (OsiriX MD, Pixmeo SARL, Bernex, Switzerland) on a Macintosh computer for further processing. The multiplanar reconstruction was used to manipulate the view of each tooth individually in the transverse, sagittal, or coronal plane to obtain images for measurement of tooth root areas (TRA). For the incisors and canines, one sagittal view of the root was chosen as this yielded the most consistent cross-section of the entire tooth root due to the rostro-caudal curvature of the incisors (Figure 1A). For the premolars and molars, a transverse view was chosen for TRA measurement due to the degree of curvature in the transverse plane. The transverse view enabled the most consistent and reproducible cross-sectional view of the entire tooth root (Figure 1B). Two images of each tooth were taken for premolars and molars with more than one root, one transverse image of each of the mesial and distal roots, respectively. Each image provides one TRA measurement site.

Table 1. Analysis of Yucatan minipigs' dental data based on their gender, age, and tooth loss

Animal	Sex	Age (year)	Number of missing teeth	Missing teeth
1	F	2	6/44	103, 105, 111, 203, 205, 211
2	F	2	4/44	105, 111, 205, 211
3	F	2	5/44	105, 107, 111, 205, 211
4	F	2	3/44	105, 205, 403
5	F	2	6/44	105, 111, 205, 211, 303, 403
6	F	2	6/44	105, 111, 205, 211, 311, 411
7	F	3	2/44	105, 205
8	M	6	5/44	103, 105, 203, 205, 406
9	M	6	2/44	105, 205
10	M	6	4/44	109, 209, 306, 409
11	M	6	8/44	103, 106, 303, 305, 309, 310, 405, 410
12	M	6	13/44	107-111, 205, 206, 208, 209, 305, 306, 405, 409
13	M	6	3/44	105, 309, 409
14	M	6	14/44	103, 105, 109, 203, 206, 209, 301-303, 305, 309, 405, 406, 409
15	M	6	6/44	105, 109, 205, 401, 406, 411
16	M	6	6/44	105, 205, 305, 306, 405, 409
17	F	7	17/44	103, 105-110, 203, 205-210, 305, 309, 405
18	F	9	21/44	102, 103, 105-110, 203, 205-210, 305, 310, 405-408, 410
19	F	10	26/44	103, 105, 107-111, 203, 205-211, 308-311, 405-411
20	F	13	10/44	103, 105, 109, 110, 202, 205, 208-210, 409

F: Female, M: Castrated male

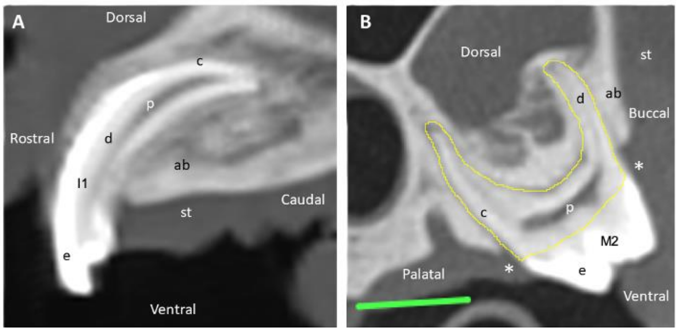

Figure 1. The CT images of a representative incisor (A) and molar (B). Enamel (e) and cementum (c) are bright white. Dentin (d) and alveolar bone (ab) are light grey. Soft tissue (st) and tooth root pulp (p) are darker grey. A: Mandibular first incisor (I1), sagittal view showing the uneven distribution of enamel, with most of the enamel on the rostral surface. B: Maxillary second molar (M2), mesial transverse view. The green bar is the scale bar (1cm) for scale calibration. The tooth root area (outlined in yellow) was calculated by tracing the tooth root outline from the buccal to the palatal/lingual cemento-enamel junction (*).

The TRA was measured two-dimensionally at each TRA measurement site by two different observers using a freeware image processing program, Image J. First, the scale was calibrated for each image individually using the scale bar incorporated into the image (Figure 1B, green bar). Then the circumference of the tooth roots on each image was traced freehand from the buccal to the lingual/palatal cemento-enamel junction (CEJ; Figure 1B, yellow outlines). The area was calculated using an internal algorithm provided by the ImageJ software. Three consecutive TRA measurements were made for each image by each observer.

2.6. Calculation of weighting factors

Using the data from the healthy pig CTs and derived from a previous study[12], a weighting factor was calculated for each LOC evaluated for gingival bleeding and periodontal pocket depth. The mean tooth root area (TRA_{MEAN}) for each measurement site was calculated from all TRA measurements made at each site in both pigs by both observers (Table 2). Each TRA_{MEAN} was then divided by the number of LOC (2 per site) to generate a TRA for each LOC (TRA_{LOC}).

$$TRA_{LOC} = \frac{TRA_{MEAN}}{2}$$

TRA_{TOTAL} was calculated as the sum of all TRA_{MEAN} values for all sites. Each TRA_{LOC} was divided by TRA_{TOTAL} to give a weighting factor for each LOC (LOC_{WT}), which represented the contribution of each TRA_{LOC} expressed as a proportion of TRA_{TOTAL} (Table 3).

$$LOC_{WT} = \frac{TRA_{LOC}}{TRA_{TOTAL}}$$

The sum of all the weighting factors was 1. All measured TRAs and calculated TRA_{MEAN}, TRA_{LOC}, and LOC_{WT} are available in supplementary tables 2 and 3.

Table 2. The mean tooth root areas from each measurement site in two healthy pigs, taken by two different observers, and used to calculate the weighting factors

Tooth	View	PIG 6 OBS 1 TRA1 (cm²)	PIG 6 OBS 1 TRA2 (cm²)	PIG 6 OBS 1 TRA3 (cm²)	PIG 6 OBS 2 TRA1 (cm²)	PIG 6 OBS 2 TRA2 (cm²)	PIG 6 OBS 2 TRA3 (cm²)	PIG 7 OBS 1 TRA1 (cm²)	PIG 7 OBS 1 TRA2 (cm²)	PIG 7 OBS 1 TRA3 (cm²)	PIG 7 OBS 2 TRA1 (cm²)	PIG 7 OBS 2 TRA2 (cm²)	PIG 7 OBS 2 TRA3 (cm²)	TRAMEAN (cm²)
101	S	1.40	1.46	1.44	1.60	1.67	1.61	1.53	1.42	1.36	1.87	1.94	1.90	1.60
102	S	0.57	0.57	0.58	0.45	0.44	0.46	0.42	0.41	0.43	0.40	0.48	0.42	0.47
103	S	NA	NA	NA	NA	NA	NA	0.38	0.38	0.38	0.36	0.37	0.37	0.37
104	S	1.44	1.41	1.18	1.44	1.47	1.44	1.09	1.05	1.05	1.31	1.32	1.32	1.29
105	S	NA	NA	NA	NA	NA	NA	0.25	0.23	0.25	0.32	0.34	0.32	0.29
106	TD	0.40	0.40	0.42	0.43	0.46	0.43	0.35	0.35	0.41	0.45	0.43	0.45	0.41
106	TM	0.44	0.44	0.43	0.36	0.36	0.37	0.33	0.35	0.34	0.38	0.39	0.39	0.38
107	TD	0.58	0.57	0.55	0.57	0.58	0.58	0.46	0.46	0.57	0.55	0.53	0.53	0.54
107	TM	0.64	0.64	0.62	0.67	0.64	0.65	0.39	0.35	0.44	0.70	0.68	0.67	0.59
108	TD	0.82	0.84	0.80	0.73	0.73	0.75	0.62	0.61	0.57	0.78	0.76	0.77	0.73
108	TM	0.64	0.66	0.67	0.70	0.72	0.70	0.64	0.61	0.62	0.89	0.89	0.87	0.72
109	TD	1.03	1.11	1.07	1.15	1.16	1.11	1.10	1.09	1.08	1.09	1.07	1.03	1.09
109	TM	0.99	1.05	1.05	1.01	0.99	1.05	1.01	1.00	1.08	1.06	1.04	1.06	1.03
110	TM	1.10	1.08	1.10	1.22	1.25	1.22	1.19	1.18	1.19	1.41	1.42	1.47	1.23
110	TD	0.45	0.43	0.45	0.37	0.41	0.41	1.12	1.10	1.10	1.09	1.13	1.02	0.76
111	TD	1.19	1.18	1.15	0.98	1.17	1.26	0.73	0.73	0.71	0.77	0.79	0.74	0.95
111	TM	1.16	1.16	1.19	1.16	1.14	1.14	1.17	1.15	1.20	1.13	1.12	1.12	1.15
201	S	1.48	1.43	1.50	1.62	1.67	1.63	1.14	1.39	1.31	1.45	1.57	1.55	1.48
202	S	0.42	0.43	0.46	0.58	0.59	0.58	0.43	0.43	0.43	0.55	0.52	0.48	0.49
203	S	NA	NA	NA	NA	NA	NA	0.37	0.36	0.37	0.41	0.43	0.45	0.40
204	S	0.89	0.89	0.90	1.14	1.05	1.12	1.11	1.12	1.09	1.22	1.24	1.16	1.08
205	S	NA	NA	NA	NA	NA	NA	0.21	0.22	0.17	0.27	0.26	0.26	0.23
206	TD	0.27	0.28	0.28	0.32	0.31	0.32	0.28	0.28	0.30	0.39	0.37	0.38	0.32
206	TM	0.28	0.30	0.29	0.38	0.35	0.38	0.60	0.62	0.62	0.39	0.39	0.39	0.41
207	TD	0.42	0.42	0.42	0.53	0.56	0.51	0.62	0.62	0.61	0.54	0.51	0.52	0.52
207	TM	0.62	0.62	0.62	0.80	0.83	0.81	0.66	0.65	0.67	0.60	0.64	0.61	0.68
208	TD	0.67	0.68	0.71	0.78	0.75	0.73	0.67	0.66	0.67	0.65	0.68	0.69	0.69
208	TM	0.85	0.89	0.86	0.76	0.75	0.76	0.64	0.63	0.62	0.63	0.60	0.59	0.72
209	TD	1.08	1.08	1.09	1.22	1.22	1.27	1.08	1.10	1.06	1.21	1.20	1.18	1.15
209	TM	1.06	1.03	1.07	0.99	0.99	0.99	1.25	1.30	1.26	1.17	1.18	1.19	1.12
210	TD	1.26	1.23	1.29	1.15	1.16	1.16	1.45	1.43	1.34	1.33	1.43	1.35	1.30
210	TM	1.29	1.23	1.30	1.21	1.17	1.19	1.27	1.30	1.28	1.27	1.14	1.17	1.24
211	TD	NA	NA	NA	NA	NA	NA	0.71	0.68	0.71	0.72	0.80	0.93	0.76
211	TM	NA	NA	NA	NA	NA	NA	1.32	1.34	1.38	1.36	1.30	1.38	1.35
301	S	1.66	1.62	1.64	1.39	1.34	1.36	0.62	0.61	0.60	0.73	0.76	0.74	1.09
302	S	1.36	1.36	1.36	1.69	1.64	1.71	0.52	0.52	0.54	0.52	0.53	0.57	1.03
303	S	0.51	0.51	0.52	0.51	0.54	0.55	0.31	0.32	0.32	0.33	0.35	0.35	0.43
304	S	1.52	1.52	1.62	1.51	1.49	1.52	1.59	1.59	1.63	1.75	1.72	1.70	1.60
305	S	0.26	0.25	0.25	0.32	0.32	0.30	0.23	0.23	0.22	0.27	0.29	0.28	0.27
306	TD	0.24	0.24	0.25	0.34	0.33	0.33	0.41	0.42	0.41	0.51	0.53	0.53	0.38
306	TM	0.31	0.30	0.32	0.38	0.41	0.41	0.30	0.31	0.31	0.36	0.36	0.36	0.34
307	TD	0.35	0.35	0.36	0.39	0.40	0.40	0.32	0.34	0.32	0.39	0.43	0.39	0.37
307	TM	0.33	0.33	0.31	0.37	0.37	0.38	0.37	0.38	0.37	0.53	0.54	0.51	0.40
308	TD	0.49	0.50	0.49	0.48	0.44	0.44	0.48	0.51	0.48	0.50	0.43	0.44	0.47
308	TM	0.61	0.62	0.62	0.65	0.64	0.64	0.87	0.83	0.89	1.04	1.02	0.92	0.78
309	TD	0.87	0.86	0.86	0.85	0.88	0.88	0.74	0.75	0.74	0.70	0.71	0.73	0.80
309	TM	0.72	0.73	0.72	0.72	0.70	0.71	0.85	0.86	0.83	0.93	0.99	0.95	0.81
310	TD	1.08	1.10	1.06	1.09	1.06	1.05	1.05	1.00	0.98	0.87	0.89	0.87	1.01
310	TM	1.39	1.40	1.37	1.42	1.37	1.35	1.05	1.07	1.05	1.11	1.12	0.99	1.22
311	TD	1.01	1.01	0.98	0.99	1.00	0.93	0.69	0.68	0.69	1.14	1.07	1.10	0.94
311	TM	1.06	1.06	1.07	1.01	0.92	0.96	1.10	1.01	1.02	1.10	1.05	1.02	1.03
401	S	1.50	1.49	1.47	1.69	1.69	1.66	1.82	1.82	1.78	1.74	1.80	1.75	1.68
402	S	1.63	1.54	1.55	1.62	1.69	1.70	1.15	1.15	1.16	1.03	1.03	1.02	1.35
403	S	0.55	0.55	0.53	0.55	0.59	0.58	0.62	0.62	0.62	0.46	0.42	0.43	0.54
404	S	2.01	2.02	2.07	1.08	1.07	0.91	1.16	1.13	1.15	1.57	1.59	1.58	1.44
405	S	0.23	0.23	0.20	0.26	0.26	0.28	0.22	0.22	0.23	0.34	0.32	0.33	0.26
406	TD	0.28	0.26	0.47	0.31	0.30	0.30	0.43	0.44	0.44	0.55	0.56	0.56	0.41
406	TM	0.24	0.24	0.26	0.32	0.35	0.34	0.42	0.42	0.42	0.49	0.47	0.47	0.37
407	TD	0.27	0.29	0.27	0.62	0.63	0.64	0.59	0.56	0.55	0.54	0.58	0.56	0.51
407	TM	0.57	0.59	0.56	0.61	0.58	0.63	0.27	0.28	0.26	0.31	0.27	0.30	0.44
408	TD	0.52	0.52	0.49	0.53	0.51	0.49	0.45	0.37	0.45	0.52	0.57	0.55	0.50
408	TM	0.47	0.47	0.49	0.70	0.69	0.69	0.51	0.50	0.50	0.51	0.51	0.50	0.54
409	TD	0.82	0.81	0.83	0.88	0.87	0.91	0.73	0.73	0.75	0.76	0.74	0.72	0.80
409	TM	0.99	1.00	0.99	0.98	0.98	0.90	0.94	0.95	0.95	0.84	0.82	0.82	0.93
410	TD	1.06	1.07	1.07	1.03	1.06	1.02	0.99	1.04	1.05	0.94	1.00	1.02	1.03
410	TM	1.26	1.23	1.25	1.21	1.18	1.22	1.16	1.16	1.17	1.05	1.10	1.12	1.18
411	TD	0.80	0.81	0.82	0.75	0.75	0.71	0.88	0.88	0.85	0.87	0.84	0.81	0.81
411	TM	1.28	1.29	1.29	1.15	1.14	1.19	1.09	0.99	1.04	1.18	1.18	1.22	1.17
													TRATOTAL	54.45

S: Sagittal, TM: Transverse medial, TD: Transverse distal, TRA: Tooth root area, OBS: observer, NA: Not assessed.

Table 3. Weighting factors for each measurement location that can be used to implement the total mouth periodontitis scoring system

Tooth	View	TRA_{MEAN} (cm²)	LOC		TRA_{LOC} (cm²)	LOC_{WT}
101	S	1.60	101	buc	0.80	0.015
			101	pal	0.80	0.015
102	S	0.47	102	buc	0.23	0.004
			102	pal	0.23	0.004
103	S	0.37	103	buc	0.19	0.003
			103	pal	0.19	0.003
104	S	1.29	104	buc	0.65	0.012
			104	pal	0.65	0.012
105	S	0.29	105	buc	0.14	0.003
			105	pal	0.14	0.003
106	TM	0.38	106	M buc	0.19	0.004
			106	M pal	0.19	0.004
106	TD	0.41	106	D buc	0.21	0.004
			106	D pal	0.21	0.004
107	TM	0.59	107	M buc	0.30	0.005
			107	M pal	0.30	0.005
107	TD	0.54	107	D buc	0.27	0.005
			107	D pal	0.27	0.005
108	TM	0.72	108	M buc	0.36	0.007
			108	M pal	0.36	0.007
108	TD	0.73	108	D buc	0.37	0.007
			108	D pal	0.37	0.007
109	TM	1.03	109	M buc	0.52	0.009
			109	M pal	0.52	0.009
109	TD	1.09	109	D buc	0.54	0.010
			109	D pal	0.54	0.010
110	TM	1.23	110	M buc	0.62	0.011
			110	M pal	0.62	0.011
110	TD	0.76	110	D buc	0.38	0.007
			110	D pal	0.38	0.007
111	TM	1.15	111	M buc	0.58	0.011
			111	M pal	0.58	0.011
111	TD	0.95	111	D buc	0.47	0.009
			111	D pal	0.47	0.009
201	S	1.48	201	buc	0.74	0.014
			201	pal	0.74	0.014
202	S	0.49	202	buc	0.25	0.005
			202	pal	0.25	0.005
203	S	0.40	203	buc	0.20	0.004
			203	pal	0.20	0.004
204	S	1.08	204	buc	0.54	0.010
			204	pal	0.54	0.010
205	S	0.23	205	buc	0.12	0.002
			205	pal	0.12	0.002
206	TM	0.41	206	M buc	0.21	0.004
			206	M pal	0.21	0.004
206	TD	0.32	206	D buc	0.16	0.003
			206	D pal	0.16	0.003
207	TM	0.68	207	M buc	0.34	0.006
			207	M pal	0.34	0.006
207	TD	0.52	207	D buc	0.26	0.005
			207	D pal	0.26	0.005
208	TM	0.72	208	M buc	0.36	0.007
			208	M pal	0.36	0.007
208	TD	0.69	208	D buc	0.35	0.006
			208	D pal	0.35	0.006
209	TM	1.12	209	M buc	0.56	0.010
			209	M pal	0.56	0.010
209	TD	1.15	209	D buc	0.57	0.011
			209	D pal	0.57	0.011
210	TM	1.24	210	M buc	0.62	0.011
			210	M pal	0.62	0.011
210	TD	1.30	210	D buc	0.65	0.012
			210	D pal	0.65	0.012
211	TM	1.35	211	M buc	0.67	0.012
			211	M pal	0.67	0.012
211	TD	0.76	211	D buc	0.38	0.007
			211	D pal	0.38	0.007
301	S	1.09	301	buc	0.54	0.010
			301	ling	0.54	0.010
302	S	1.03	302	buc	0.51	0.009

			302	ling	0.51	0.009
303	S	0.43	303	buc	0.21	0.004
			303	ling	0.21	0.004
304	S	1.60	304	buc	0.80	0.015
			304	ling	0.80	0.015
305	S	0.27	305	buc	0.13	0.002
			305	ling	0.13	0.002
306	TM	0.34	306	M buc	0.17	0.003
			306	M ling	0.17	0.003
306	TD	0.38	306	D buc	0.19	0.003
			306	D ling	0.19	0.003
307	TM	0.40	307	M buc	0.20	0.004
			307	M ling	0.20	0.004
307	TD	0.37	307	D buc	0.18	0.003
			307	D ling	0.18	0.003
308	TM	0.78	308	M buc	0.39	0.007
			308	M ling	0.39	0.007
308	TD	0.47	308	D buc	0.24	0.004
			308	D ling	0.24	0.004
309	TM	0.81	309	M buc	0.41	0.007
			309	M ling	0.41	0.007
309	TD	0.80	309	D buc	0.40	0.007
			309	D ling	0.40	0.007
310	TM	1.22	310	M buc	0.61	0.011
			310	M ling	0.61	0.011
310	TD	1.01	310	D buc	0.50	0.009
			310	D ling	0.50	0.009
311	TM	1.03	311	M buc	0.52	0.009
			311	M ling	0.52	0.009
311	TD	0.94	311	D buc	0.47	0.009
			311	D ling	0.47	0.009
401	S	1.68	401	buc	0.84	0.015
			401	ling	0.84	0.015
402	S	1.35	402	buc	0.68	0.012
			402	ling	0.68	0.012
403	S	0.54	403	buc	0.27	0.005
			403	ling	0.27	0.005
404	S	1.44	404	buc	0.72	0.013
			404	ling	0.72	0.013
405	S	0.26	405	buc	0.13	0.002
			405	ling	0.13	0.002
406	TM	0.37	406	M buc	0.19	0.003
			406	M ling	0.19	0.003
406	TD	0.41	406	D buc	0.20	0.004
			406	D ling	0.20	0.004
407	TM	0.44	407	M buc	0.22	0.004
			407	M ling	0.22	0.004
407	TD	0.51	407	D buc	0.25	0.005
			407	D ling	0.25	0.005
408	TM	0.54	408	M buc	0.27	0.005
			408	M ling	0.27	0.005
408	TD	0.50	408	D buc	0.25	0.005
			408	D ling	0.25	0.005
409	TM	0.93	409	M buc	0.46	0.009
			409	M ling	0.46	0.009
409	TD	0.80	409	D buc	0.40	0.007
			409	D ling	0.40	0.007
410	TM	1.18	410	M buc	0.59	0.011
			410	M ling	0.59	0.011
410	TD	1.03	410	D buc	0.51	0.009
			410	D ling	0.51	0.009
411	TM	1.17	411	M buc	0.58	0.011
			411	M ling	0.58	0.011
411	TD	0.81	411	D buc	0.41	0.007
			411	D ling	0.41	0.007
SUM		54.45		SUM	54.45	1.00

S: Sagittal, TM: Transverse medial, TD: Transverse distal, buc: Buccal, pal: Palatal, ling: Lingual, LOC: Location, TRA: Tooth root area, TRA_{LOC}: Tooth root area of each measurement location, LOC_{WT}: Weighting factor for each measurement location.

2.7. Calculation of total mouth periodontitis FULLSCORE

The weighting factors were used to calculate a total mouth periodontal score for gingival bleeding (TMPS-G) and periodontal pocket depth (TMPS-P) for each animal. A weighted TMPS-G/TMPS-P was computed using all measurements made at all LOC, for incisors; buccal and palatal/lingual, for premolars and molars; medial buccal, distal buccal, mesial palatal/lingual, and distal palatal/lingual, for each animal (FULLSCORE). The TMPS-G or TMPS-P at each measurement LOC was multiplied by the corresponding LOC_{WT}. Then all TMPS-G and TMPS-P were summed, respectively, to result in one TMPS-G FULLSCORE and one TMPS-P FULLSCORE for each animal. Missing teeth were accounted for by adjusting the weighting factors to the total number of teeth present in the oral cavity for each animal. The TMPS-G and TMPS-P were calculated using only teeth that were present.

2.8. Calculation of total mouth periodontitis QUICKSCORE

To assess whether a shortened dental exam with a subset of teeth (QUICKSCORE) can replace a full dental exam (FULLSCORE), the TMPS-G and TMPS-P were calculated using only the specified locations. Right maxillary and mandibular buccal locations of I3, C, P3, P4, M1, M2, or 103, 104, 107, 108, 109, 110, 403, 404, 407, 408, 409, and 410. The weighting factor for each location was adjusted to the reduced number of total teeth before calculation of the QUICKSCORE TMPS-G and TMPS-P according to the formulae provided by Harvey et al.[12,13].

2.9. Statistical analysis

All statistical analyses were performed using STATA Statistical Software version 14 (StataCorp LLC, College Station, TX, USA). The Lin's concordance correlation coefficient (CCC)[17] was used to evaluate the concordance between FULLSCORE and the QUICKSCORE for both TMPS-G and TMPS-P. The interclass correlation coefficient (ICC) was used to assess intra- and inter-observer correlation of measurements to develop the weighting factors. The interpretation of ICC values was a range of 0.41 to 0.60, indicating moderate concordance, 0.61 to 0.80, reflecting substantial concordance, and 0.81 to 1.00 represented excellent concordance[18]. Spearman correlation was used to compare TMPS-G and TMPS-P with age, LOCs% with GB > 0, LOCs% with PD > 3mm, and mean GB and mean PD with TMPS-G and TMPS-P, respectively. The level of significance was set to 5% ($p < 0.05$).

3. Results

3.1. Intra- and inter-observer concordance for tooth root measurements

A total of 130 images were acquired from pigs 6 and 7. For pig 7, 68 images were acquired from a complete set of 44 teeth. Pig 6 was missing the maxillary third incisors and first premolars (103, 203, 105, 205) and had one unerupted molar (211). Therefore, only 62 images from 39 teeth could be obtained for pig 6. Three TRA measurements were made per image, yielding a total of 780 measurements for evaluation (Table 2). The ICC (95% CI) for three separate TRA measurements at each LOC was 0.92 (0.90-0.94). The inter-observer correlation, comparing measurements between different observers, was 0.96 (0.95-0.97). Since the agreement among observers' measurements was very high, the average of all six measurements for each tooth and location (TRA_{MEAN}) was used to determine the weighting factor for each LOC (LOC_{WT}; Table 3).

3.2. Clinical findings

Table 1 summarizes the clinical data for each animal. In general, the animals in the present study had a dental formula of two (I3/3, C1/1, P4/4, M3/3), as described by Wang et al.[6] for Sinclair miniature pigs. However, not all the 2-year-old animals had maxillary P1 teeth, and half (3/6) were missing mandibular/maxillary I3. Additionally, the M3 teeth were not fully erupted in many of the young animals. Although all young animals exhibited gingival bleeding upon probing, they had only mild plaque buildup and minimal gingival recession (Figure 2A). In contrast, all the 6-year-old animals (9/9) had moderate to severe plaque buildup, gingival bleeding, many missing teeth, and teeth worn down to the gum line (Figure 2B and C). All 7-year-old and older animals (4/4) had severe plaque and calculus build-up (Figure 2D), moderate-severe gingival recession, and large numbers of missing teeth. In one 7-year-old animal, no gingival bleeding scores were obtained as the oral exam was performed immediately after euthanasia due to age-related illness. Results of the oral exam, including the calculated TMPS FULLSCORES and QUICKSCORES for each animal, are summarized in Table 4.

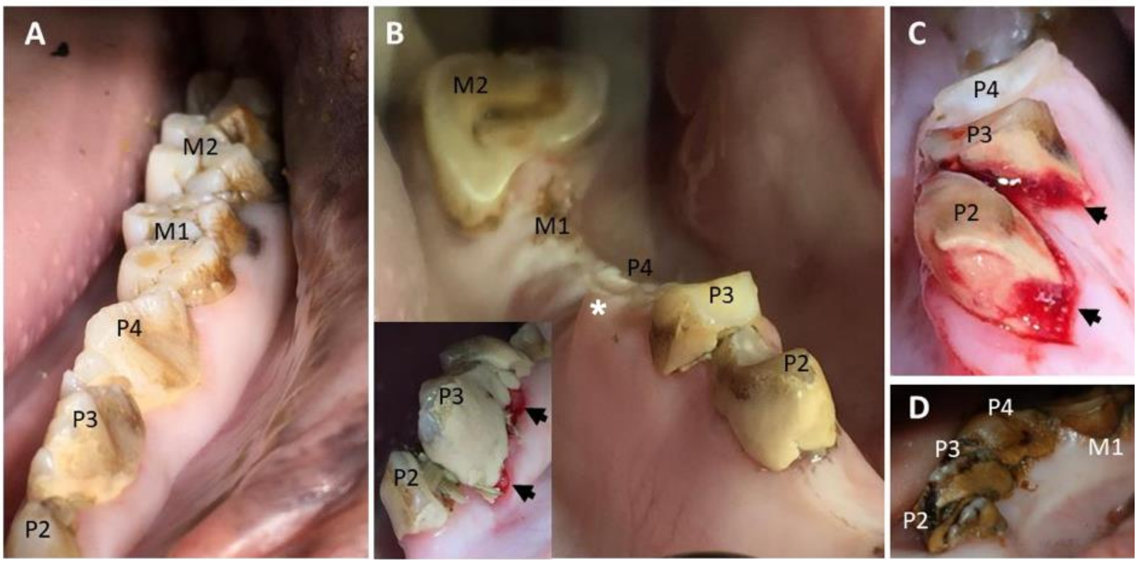

Figure 2. Mandibular premolars and molars in 2-13-year-old Yucatan Minipigs. Buccal is to the right and lingual to the left in all images. A: Mandibular premolars and molars from a 2-year-old animal showing mild plaque buildup and healthy gingiva. B and C: Mandibular teeth in a 6-year-old animal showing moderate plaque buildup, gingival bleeding (Black arrows), and missing teeth (M1) as well as teeth worn down to the gum line (*). B insert shows more severe plaque buildup and gingival bleeding (Black arrows). D: Severe plaque and calculus buildup around the premolars of a 10-year-old animal.

Table 4. Oral analysis for each Yucatan minipig

Animal	GR (0-3)	PL (0-3)	Nr LOCs	Mean GB	TMPS-G FULL	TMPS-G QUICK	LOC % GB >0	Mean PD	TMPS-P FULL	TMPS-P QUICK	LOCs % PD >3mm
1	0	1	120	1.57	1.48	2.00	80%	2.28	2.30	2.60	7%
2	0	1	124	1.77	1.68	1.22	85%	2.73	2.86	2.73	19%
3	1	1	120	1.50	1.58	1.28	75%	2.34	2.46	2.49	10%
4	0	1	130	1.14	1.14	0.93	62%	2.16	2.18	2.30	2%
5	1	1	120	1.13	0.94	1.06	57%	2.13	2.12	1.99	1%
6	1	1	116	0.97	1.01	0.77	48%	2.30	2.33	2.44	8%
7	1	1	132	1.12	1.04	1.28	56%	2.19	2.17	2.46	5%
8	1	2	124	0.71	0.63	0.79	65%	2.58	2.51	2.39	14%
9	3	3	132	1.67	1.64	1.55	89%	2.81	2.88	3.49	23%
10	2	2	120	0.15	0.20	0.04	12%	2.19	2.38	2.21	8%
11	3	3	112	0.82	0.76	0.85	82%	2.72	2.61	2.36	15%
12	3	3	90	1.71	1.68	1.44	89%	2.64	2.63	2.84	17%
13	1	2	125	0.51	0.42	0.53	51%	2.24	2.31	2.00	5%
14	1	3	96	0.73	0.74	0.50	58%	2.72	2.37	2.76	19%
15	1	3	118	0.78	0.70	0.46	71%	2.53	2.44	2.42	16%
16	2	3	120	1.55	1.52	1.63	88%	2.61	2.63	2.77	12%
17	3	3	80	NR	NR	NR	NR	4.01	4.04	3.64	61%
18	2	3	66	1.91	1.89	1.46	100%	4.88	4.76	4.76	80%
19	2	3	40	0.43	0.56	1.05	28%	4.48	4.19	5.00	68%
20	3	3	104	1.94	1.95	1.80	100%	5.05	5.14	4.27	74%

GR: Gingival recession, PL: Plaque accumulation. Scoring system for GR and PL: 0: None, 1: Mild, 2: Moderate, 3: Severe, Nr LOC: Number of locations (LOCs) evaluated, GB: Gingival bleeding, FULL: FULLSCORE, QUICK: QUICKSCORE, PD: Periodontal pocket depth, NR: Not recorded.

3.3. Correlation of total mouth periodontitis score with age and disease severity

Scatterplots are shown of TMPS-G (Figure 3A to C) and TMPS-P (Figure 3D to F) plotted against the following variables including age (Figure 3A and D), LOCs% with GB > 0 (Figure 3B), LOCs% with PD > 3mm (Figure 3E), mean GB (Figure 3C) and mean PD (Figure 3F). Age was not significantly correlated with TMPS-G but showed a significant positive correlation with TMPS-P (p=0.003). The LOCs% with GB score > 0 was positively correlated with TMPS-G (p < 0.001). The LOCs% with PD > 3mm was positively correlated with TMPS-P (p < 0.001). The mean GB and PD were positively correlated with the TMPS-G (p < 0.001) and the TMPS-P (p < 0.001), respectively.

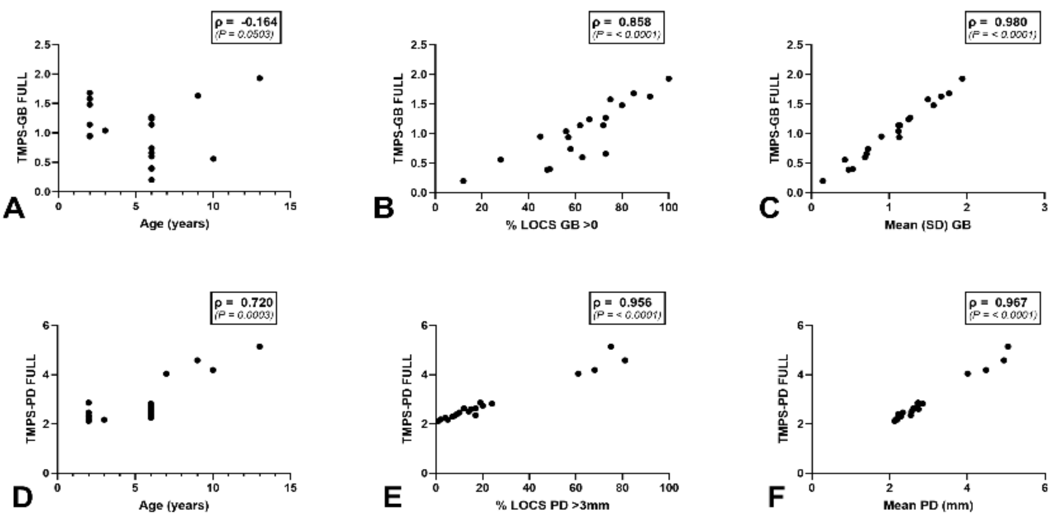

Figure 3. Scatterplots of TMPS-GB (A to C) and TMPS-PD (D to F) plotted against age (A and D). B: LOCs% with GB > 0, LOCs% with PD > 3mm, C: Mean GB, F: Mean PD. Age is not significantly correlated with TMPS-GB FULLSCORE (p = 0.0503), but it is correlated with TMPS-PD FULLSCORE (p < 0.001). TMPS-GB is significantly positively correlated with LOCs% GB > 0 and mean GB (p < 0.001). TMPS-PD is significantly positively correlated with LOCs% PD > 3mm and mean PD (p < 0.001).

3.4. Total mouth periodontitis FULLSCORE and QUICKSCORE concordance

For TMPS-G QUICKSCORE and FULLSCORE, the CCC was excellent; 0.857 (95% CI, 0.731-0.983, $p < 0.001$). Similarly, for TMPS-P QUICKSCORE and FULLSCORE, the CCC was excellent; 0.883 (95% CI, 0.781-0.985, $p < 0.001$).

4. Discussion

The observations regarding tooth eruption and dental formula align with the results reported by Wang et al.[6] concerning miniature pigs, except that all of the 2-year-old pigs (6/6) were found to be missing the third incisor, and half of them (3/6) lacked the first maxillary premolar. This discrepancy may be due to sexual dimorphism, as all the young pigs in the present study were female, or because incisors have the broadest range of eruption times in both humans and pigs[6]. The TMPS FULLSCORE and QUICKSCORE are excellently correlated. Therefore, TMPS-FULLSCORE was a tool to evaluate oral health for those who desire a comprehensive evaluation that can localize disease within the mouth. TMPS-QUICKSCORE was a practical, fast measure of overall mouth health that can be used for any type of porcine model while limiting time under general anesthesia and without needing to change positioning on the table.

A limitation in the present study was that we measured periodontal pocket depth rather than actual attachment loss of the periodontal tissues. Periodontal pocket depth was calculated as the distance from the bottom of the periodontal pocket to the gingival margin[19]. However, with gingival recession, caused by increased periodontal tissue destruction, the gingival margins can drop significantly below the level of the CEJ, where the healthy gingival margin typically resides[19]. When this recession occurred, the actual pocket depth might be small, but the amount of attachment loss was significant. Measuring the distance from the CEJ to the bottom of the periodontal pocket is a much better measure of periodontal tissue destruction[19]. Unfortunately, in the present model, using the CEJ was not possible in animals over 6 years old due to the degree of plaque, calculus buildup, and discoloration of the enamel, making it extremely difficult to identify the CEJ. Many of the premolars and molars in these pigs were worn down below the level of the CEJ, making identification and measurement of true AL impossible. Based on the current findings, 6-year-old animals subjectively had far more gingival recession than 2-year-old animals.

The TMPS-P system, developed by Harvey et al.[12], was created using actual teeth harvested from cadaver animals, which was challenging to do for miniature pigs, as it was complicated to extract premolars and molars with their delicate roots intact successfully. Thus, CT images were chosen to measure the areas of the tooth roots, which helped to acquire the necessary data without sacrificing any animals. In contrast to Harvey et al.[12], who measured the buccal or lingual/palatal tooth root surface area for each tooth, in the present study, the decision was made to measure the sagittal view of the root for incisors and canines, as well as a transverse view of the mesial and distal surfaces for premolar and molar teeth. These views provided the clearest full view of each tooth root, regardless of unevenness or curvature. As most tooth roots are curved in three dimensions, neither method provided a perfect measurement of the tooth root surface area. However, any measurement error would be the same for all roots. Therefore, the areas measured in the present study were sufficient to quantify the size of tooth roots throughout the oral cavity and can be used to determine how much each location contributes to overall periodontal disease in an individual.

Harvey et al.[12] used the circumference of the CEJ as the weighting factor for TMPS-G at each site. The CEJ was measured on an apical view of each tooth, which was not possible in the present study, as so many teeth were worn down below the CEJ. Based on the current study, pigs had a very uneven CEJ that was often not visually apparent compared to dogs and humans. Thus, the tooth root area at each site was selected as a weighting factor for both TMPS-G and TMPS-P.

The lack of correlation between the age of the animals and the TMPS-G suggested that the TMPS scores were not simply a measure of an animal's age. The TMPS-G and TMPS-P positively correlated with both the mean GB and PD, respectively, but also with the number of sites within the mouth that were severely affected by periodontal disease (LOCs% with PD > 3mm and LOCs% with GB > 0). These positive correlations supported the claim that TMPS is an acceptable indicator of comprehensive and local periodontal disease.

The QUICKSCORE enabled evaluators to score a subset of buccal teeth on one side of the mouth, which were easier to access and could be scored more quickly than the entire oral cavity. The buccal side was more accessible than the palatal side, and scoring only one side meant that there was no need to adjust the patient's laterality during the anesthesia process. Both the TMPS-G and TMPS-P QUICKSCORE exhibited a clear, strong linear correlation with the corresponding FULLSCORE. The QUICKSCORE should enable fast and accurate measurement of periodontal disease burden for comparison between animals and/or study time points. Different subsets of teeth have been previously tested for correlation in dogs, with the buccal sites of the right maxillary third incisor, canine, third premolar, and fourth premolar, and the mandibular canine, third premolar, and fourth premolar, found to have the best correlation with the full-mouth score. Additionally, these teeth represented the teeth that are required to be scored in the Veterinary Oral Health Council trials of plaque and calculus accumulation[13]. The FULLSCORE is more appropriate to monitor localized alterations in the oral cavity over time, particularly with an emphasis on oral health.

5. Conclusion

The present study successfully adapted the canine TMPS system to evaluate periodontal disease in Yucatan Minipigs, utilizing gingival bleeding and periodontal pocket depth as key metrics. Although certain limitations render the TMPS system less comprehensive than traditional human dental care standards, the TMPS is a valuable instrument for porcine models, particularly due to the established connection between oral and systemic health. Additionally, the QUICKSCORE can be used as a fast and convenient scoring system to track changes in TMPS over time. It is hoped that the adoption of this TMPS will not only provide a standardized measure of oral health in porcine studies of gingivitis and periodontitis but also underscore how periodontal disease can influence translational research results. Future studies should use the TMPS system to evaluate the effect of oral health on systemic well-being and pathology in translational animal models.

Declarations

Competing interests

The authors declared that they have no competing interests.

Authors' contributions

Amanda R Watkins was involved in data analysis and interpretation, drafting, critical revision of the manuscript, and final approval of the manuscript. Joanne E Haughan was involved in the acquisition, analysis, and interpretation of the data, as well as drafting and revising the manuscript, and final approval of the manuscript. Both authors (Amanda R Watkins and Joanne E Haughan) contributed equally as co-first-authors to the manuscript. Mallory Marschall was involved in data collection, analysis, drafting, and final approval of the manuscript. Darko Stefanovski was involved with data analysis and interpretation, drafting, and final approval of the manuscript. Thomas P Schaer was involved in the conceptualization, acquisition, analysis, and interpretation of the data, as well as writing and revising the manuscript. All authors reviewed and approved the final edition of the manuscript.

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Availability of data and materials

All datasets are available upon request from the correspondence.

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Ethical considerations

The authors have observed ethical issues, including plagiarism, double submission, and data originality.

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