NOTE
Internal Medicine

The effects of gel pad thickness on the evaluation of skin structures using ultrasonography in normal dogs

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ABSTRACT. Gel pads are commonly used in skin ultrasonography; however, the effects of their thickness are unknown. This study investigated the effects of pad thickness on measurements of skin thickness in 10 beagle dogs. Sonograms to measure neck skin thickness were captured without pads and using pads with thicknesses of 3, 5, 10, and 20 mm. Without pads, acoustic shading was observed due to air bubbles in the coupling gel. With 20-mm pads, echogenic artifacts were observed on the skin surface. Entry echo with 20-mm pads was significantly higher than with 3-mm pads. This suggests that visibility of the skin structure could be affected when a gel pad is not used or when a thick gel pad is selected.

KEY WORDS: artifact, gel pad, normal dog, skin thickness, skin ultrasonography

Ultrasonography is a reliable, painless, low-risk, and consistent tool for investigating the skin, thereby allowing real-time visual characterization of its layers. The measurement of skin thickness by ultrasonography has been reported as a reproducible method in human [1, 3, 8] and veterinary medicine [4, 7, 16, 17]. For example, in a study on humans, patients with psoriasis were shown to have a skin thickness significantly higher than that of healthy people [8]. A comparative study of skin thickness was performed in dogs of small breeds, comparing normal dogs, dogs with hyperadrenocorticism, and dogs treated with prednisolone. It showed that dogs with hyperadrenocorticism and dogs treated with prednisolone had a skin thickness that was significantly lower than that of the normal group. Hence, the use of ultrasound to measure skin thickness in small dogs may provide useful clinical information to differentiate between dogs with hyperadrenocorticism and healthy dogs [7]. Furthermore, measurements of skin thickness can be potentially used to infer not only skin diseases but also general conditions. While measuring skin thickness using ultrasonography may be useful in the diagnosis of skin diseases, a high accuracy would be required.

Gel pads (i.e., stand-off pads) are often used in ultrasound examinations of the body surface. This is because gel pads do not contain scatterers and are a medium that adheres to the skin and probe while providing good delineation of body surface structures [6, 11, 13, 14]. Although gel pads are often used in skin ultrasonography, the appropriate thickness of gel pads for skin ultrasonography is currently unknown. We hypothesized that since the skin is very thin and located at the most superficial layer, the thickness of the gel pad may affect the visibility of the skin structure, especially with thicker gel pads, which would reduce the visibility.

The purpose of this study was to investigate how the thickness of gel pads affects the ultrasonographic measurement of skin thickness in dogs.

Our study was performed with 10 beagles: 4 intact females and 6 intact males. Dogs with no history of skin diseases and no shampoos, conditioners, sprays, or lotions applied for two weeks prior to the experiment were selected for this study. The dogs ranged from 5 to 9 years of age, 9.0 to 13.1 kg in body weight, and 2 to 4 for body condition score. All dogs were considered healthy based on the results of clinical examination. In addition, the dogs were confirmed to be hydrated by a skin tent test and the capillary refill time assessment.

The study design was approved by the Rakuno Gakuen University Animal Experiment Committee (approval number: VH19B8), and the study conformed to all ethical guidelines established by this institution. Before the ultrasound examination, the spinous process of the axial vertebrae was palpated, and the third cervical vertebra level just caudal to it was furrowed. For reproducibility of the examination, the skin was marked with an oil-based pen at the site of probing.

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In the ultrasound imaging system (Arietta 70, Hitachi, Tokyo, Japan), we held the dogs in a sternal recumbent position; the hair on the dorsal neck region was shaved with a clipper to ensure that the dominant area was measured when the probe was applied. The dorsal neck skin was visualized using a 13 MHz linear probe (L64; Hitachi) on the location where the hair was removed and marked. First, normal coupling gel (Aquasonic Clear, Parker Laboratories, Fairfield, NJ, USA) was placed on the probe, without the use of a gel pad (no pad), and ultrasound images of the skin were recorded three times in B mode. One benefit of B mode was that every layer of the skin could be observed under all conditions. Next, images were recorded with pads (Echo Gel Pad, Yasojima, Kobe, Japan) with thicknesses of 3, 5, 10, or 20 mm that were attached without coupling gel. The thickness of all gel pads had already been adjusted by the manufacturer. The 3-mm pad was wrapped around the probe and affixed with a band; the 5-mm, 10-mm, and 20-mm pads were each cut to fit the probe with a knife (width, 10 mm; length, 5 mm) before being covered, together with the probe, using a condom (thickness: 0.038 mm). The acoustic impedance and damping rate of these gel pads were 1.42×10^6 (Pa · sec/m) and 3.5 dB/cm at a frequency of 5 MHz, respectively. To minimize the effect on skin thickness, the probe was placed on the skin slowly under all conditions, and the image was saved as soon as an image showing the skin structure was obtained to avoid excessive pressure. In accordance with a previous study [7], the skin layer was classified into skin thickness, entry echo (a hyperechoic line on the most superficial surface of the skin), epidermis (a hyperechoic layer on the surface of the skin), and reticular dermis (a hypoechoic layer deep in the skin). Each layer was measured from the three recorded images, and the average value was calculated (Fig. 1).

Fig. 1. B-mode ultrasound image of the dorsal neck skin. G, gel pad; ST, skin thickness; E, entry echo; EP, epidermis; R, reticular dermis; S, subcutis; M, muscle.

The obtained data was analyzed using free statistical software (R, a language and environment for statistical computing, version 3.6.0, R Foundation for Statistical Computing, Vienna, Austria) for the Shapiro-Wilk normality test and commercially available software (Statmate III, ATMS, Tokyo, Japan) for the one-way analysis of variance (ANOVA) and the Kruskal–Wallis test. The one-way ANOVA or Kruskal-Wallis test with Tukey's range test was used to determine the significance of the differences in thickness among each condition; significance was considered $P<0.05$. For the entry echo values (3 mm and 5 mm), a non-parametric test (Kruskal-Wallis test) was used because they were not normally distributed. For all other measurements, a parametric test (one-way ANOVA) was used.

The resulting B-mode images are shown in Fig. 2. When no pad was used, air bubbles were present in the coupling gel, resulting in acoustic shading. This phenomenon was observed in all cases when no pad was used (100%), but not in cases when the 3-, 5-, 10-, and 20-mm gel pads were used (0%, 0%, 0%, and 0%, respectively). On the other hand, echogenic artifacts were observed on the skin surface in all cases when the 20-mm pad was used (100%). However, it occurred in 10% of the cases when the 10-mm gel pad was used, but not when no pad or the 3-mm and 5-mm pads were used (0%, 0%, and 0%, respectively).

Each measurement is listed in Table 1. For skin thickness, the highest value (3.74 ± 0.96 mm) was observed when no pad was used, and the lowest value (3.17 ± 0.82 mm) was observed when the 3-mm gel pad was used; nevertheless, there was no significant difference between the conditions. For entry echo, the highest value (0.76 ± 0.22 mm) was found when no pad was used, the second highest value (0.75 ± 0.12 mm) when a 20-mm pad was used, and the lowest value when a 3-mm pad was used; in this case, there was a significant difference between the 3-mm gel pad and the 20-mm gel pad. There were no significant differences between the conditions for epidermis and reticular dermis.

In veterinary medicine, coupling gel is conventionally used in skin ultrasonography [4, 7, 16, 17]. In this study, the coupling gel was carefully placed on the probe, but the bubbles in the coupling gel could not be completely removed, and as a result, the artifacts caused by the bubbles affected the observation of the skin layer. Conversely, the images obtained in this study with each thickness of gel pads were able to visualize each skin layer well. The significant difference in entry echo while using the 3-mm versus 20-mm pads may be due to echogenic artifacts that occurred on the skin surface when the 20-mm pad was used. These artifacts are caused by the second lobe (side lobe, grating lobe) artifact, which is caused by the reflection of the second lobe on a strong reflector [2, 10]. This artifact is more likely to occur when the second lobes and reflective surfaces are orthogonal, and is often observed in organs with curved reflective surfaces, such as the bladder and diaphragm [2, 10]. In the present study, the 20-mm pad showed a tilt and curvature of the skin interface at the edge of the screen due to the buckling of the gel (Fig. 2). The second lobe artifact that occurred when the 20-mm pad was used was likely due to the formation of the slope, which caused the anti-slope to be perpendicular to the second lobes. Entry echo when no pad was used was thicker than the entry echo when a 3-mm
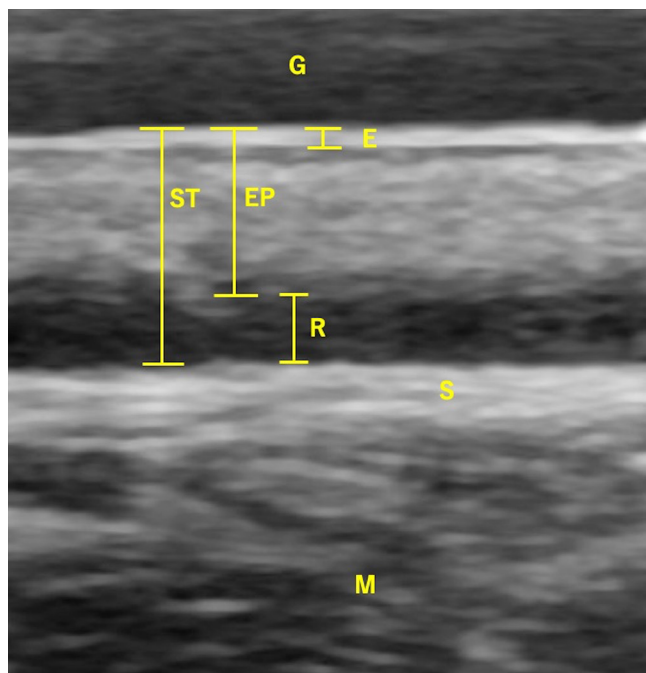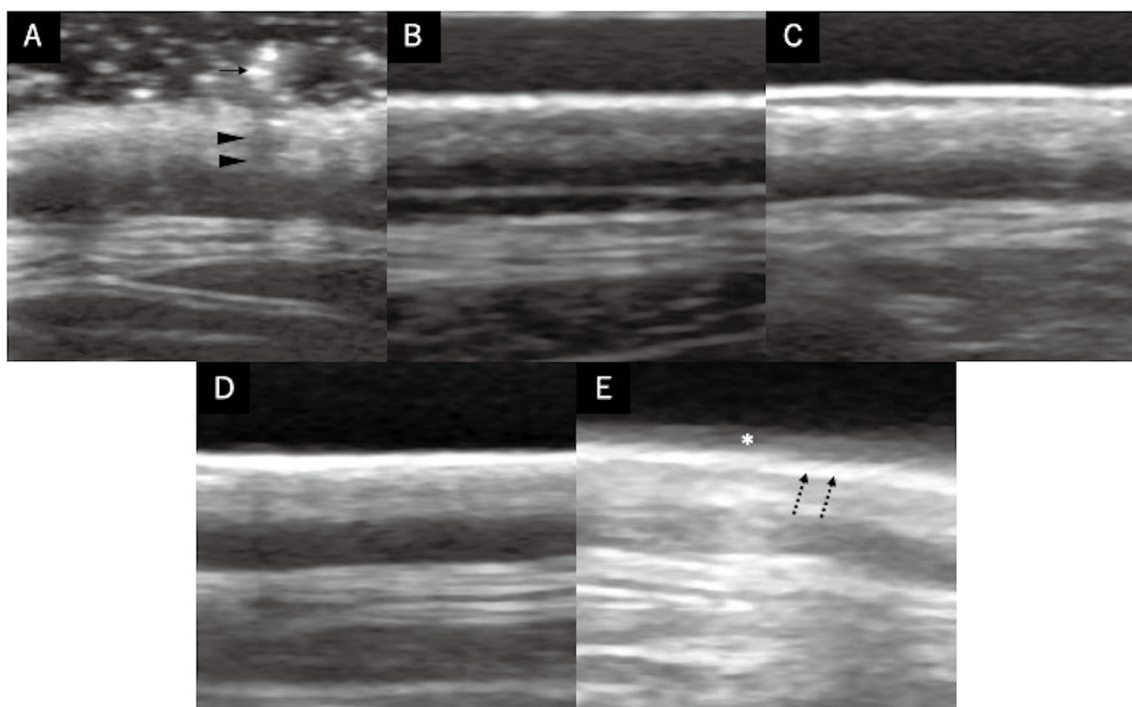
Fig. 2. Ultrasound images of the dorsal neck skin when no pad **(A)** was used, or when a 3-mm pad **(B)**, 5-mm pad **(C)**, 10-mm pad **(D)**, or 20-mm pad **(E)** was used. When no pad was used, an acoustic shadow (arrowheads) due to air bubbles (arrow) was observed **(A)**. When a 20-mm pad was used, an echogenic artifact (asterisk) and slope (dotted arrows) were observed on the skin surface **(E)**.

Table 1. The skin thicknesses at each condition

		Ultrasound-measured values				
	Gel pad	Mean (mm)	SD	Median (mm)	Maximum (mm)	Minimum (mm)
Skin thickness	No pad	3.74	0.96	3.37	5.17	2.43
	3-mm pad	3.17	0.82	3.05	4.67	1.90
	5-mm pad	3.37	0.95	3.02	4.80	2.03
	10-mm pad	3.35	0.81	3.12	4.87	2.17
	20-mm pad	3.39	0.69	3.5	4.07	2.20
Entry echo	No pad	0.76	0.22	0.83	1.03	0.40
	3-mm pad*	0.56	0.19	0.5	0.97	0.37
	5-mm pad	0.64	0.22	0.57	1.25	0.50
	10-mm pad	0.65	0.11	0.65	0.80	0.50
	20-mm pad*	0.75	0.12	0.77	0.97	0.53
Epidermis	No pad	2.17	0.45	2.00	2.73	1.40
	3-mm pad	1.88	0.42	1.92	2.37	1.40
	5-mm pad	2.04	0.48	2.10	2.80	1.40
	10-mm pad	2.04	0.46	2.02	3.03	1.40
	20-mm pad	2.22	0.38	2.28	2.70	1.30
Reticular dermis	No pad	1.61	0.64	1.52	2.45	0.47
	3-mm pad	1.28	0.46	1.17	2.07	0.47
	5-mm pad	1.36	0.56	1.35	2.17	0.37
	10-mm pad	1.30	0.45	1.38	1.83	0.40
	20-mm pad	1.23	0.50	1.15	1.93	0.30

* 3-mm pad vs. 20-mm pad, $P<0.05$. SD, standard deviation.

pad was used, although no significant difference was found. In the absence of a gel pad, the accumulation of highly echogenic bubbles in the coupling gel on the skin surface may have obscured the boundary with the entry echo and caused the overestimation. In addition, the bubbles in the coupling gel formed acoustic shadows beneath them, which obscured the layered structure of the

skin. The entry echo results from the difference in acoustic impedance between the gel and the stratum corneum of the skin surface, and its echogenicity depends on the thickness of the stratum corneum and the amount of air between the keratotic scales [4, 7, 12]. Additionally, the change in entry echo thickness is not only an artifact, but also has clinical significance in human patients with psoriasis and scleroderma, showing a significant increase compared to that in healthy subjects [5, 9], thereby reflecting pathological changes. Although gel pad thickness did not significantly alter skin thickness measurements in this study, significant differences in entry echo measurements due to gel pad thickness may have an impact in assessing skin pathological changes. As a result of these observations, it can be inferred that various artifacts could be avoided by using a thin gel pad in skin ultrasonography.

The main limitation of this study was the small number of cases. Only beagles were included in the study; thus, breed differences were not taken into account. It was also suggested that suitable gel pad thickness may vary based on the components and shape of the gel pad and ultrasound device. In addition, it cannot be denied that the use of cut gel pads may have affected the stability of the probe with the skin, especially in thicker gel pads (20 mm). Furthermore, in this study, condoms were used to cover the 5-, 10-, and 20-mm pads. Although condoms are used as a cushioning material that does not affect the image quality of ultrasound images [15], the effect of its slightly additional thickness and material difference cannot be completely excluded. Also, we tried to minimize the pressure applied to the skin by the probe so that it would not affect the skin thickness measurement, but since we were not able to quantify this pressure, we could not evaluate its consistency under each condition. Finally, inter-examiner error was not considered in this study.

In conclusion, for skin ultrasonography, although the presence or absence of a gel pad and the thickness of the gel pad do not significantly affect skin thickness measurements, the visibility of the skin structure could be affected by artifacts when no gel pad or a thick gel pad is used.

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