Effects of Angle of Epiglottis on Aerodynamic and Acoustic Parameters in Excised Canine Larynges

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Summary: Objectives. The aim of this study is to explore the effects of the angle of epiglottis (Aepi) on phonation and resonance in excised canine larynges.

Methods. The anatomic Aepi was measured for 14 excised canine larynges as a control. Then, the Aepis were manually adjusted to 60° and 90° in each larynx. Aerodynamic and acoustic parameters, including mean flow rate, sound pressure level, jitter, shimmer, fundamental frequency (F0), and formants ($F1'-F4'$), were measured with a subglottal pressure of 1.5 kPa. Simple linear regression analysis between acoustic and aerodynamic parameters and the Aepi of the control was performed, and an analysis of variance comparing the acoustic and aerodynamic parameters of the three treatments was carried out.

Results. The results of the study are as follows: (1) the larynges with larger anatomic Aepi had significantly lower jitter, shimmer, formant 1, and formant 2; (2) phonation threshold flow was significantly different for the three treatments; and (3) mean flow rate and sound pressure level were significantly different between the 60° and the 90° treatments of the 14 larynges.

Conclusions. The Aepi was proposed for the first time in this study. The Aepi plays an important role in phonation and resonance of excised canine larynges.

Key Words: Excised canine larynges—Angle of epiglottis—Aerodynamics—Acoustics—Phonation threshold flow.

INTRODUCTION

The epiglottis is a leaf-shaped cartilage that connects with the inner surface of thyroid cartilage above the anterior commissure of vocal folds. It folds down and covers the laryngeal inlet when making a swallowing motion.[1] Currently, most studies on the epiglottis concentrate on deglutition and respiration. To the best of our knowledge, little research has been done on the phonatory and resonant functions of the epiglottis. Sundberg and Titze proposed that an alteration in the dimensions of supraglottic cavities contributes to production of the singer's formant and resonant voice.[2,3] A study performed by Yanagisawa et al suggested that the speaker and singer can adjust the position and shape of their epiglottis to produce different voice qualities such as sob and opera.[4] It is common to focus on the complex structures of the vocal folds when talking about the vocal mechanism, but the analysis is flawed if it fails to include the contribution of supraglottic vocal tract resonance.[5]

The Department of Speech Pathology and Audiology at the University of Iowa has done a series of studies on supraglottic structures such as the epiglottis, false vocal folds (FVFs), and laryngeal ventricles. Alipour et al removed both the FVFs and the epiglottis and compared the acoustic and aerodynamic parameters before and after the operation. They proposed that the FVFs and epiglottis raise the resistance of airflow in the glottis and the vocal intensity in a lower frequency range (50−100 Hz).[6] In a separate study, Alipour and Finnegan investigated the supraglottic structure of eight excised canine larynges. They reported that the excised canine larynges oscillated with a significantly higher sound pressure level (SPL) with supraglottic structures included and oscillated at a significantly lower phonatory threshold pressure (PTP)[7] with supraglottic structures removed.

Finnegan et al performed an experiment on three excised canine larynges and found that (1) a decrease in subglottal pressure and glottal resistance led to an increase in mean flow rate (MFR) and fundamental frequency (F0) when the epiglottis was raised from a horizontal position to an upright position; (2) the presence of the epiglottis augmented the second partial of the acoustic signal as a resonator; and (3) the absence of the epiglottis would enhance the noise in low frequencies (0−300 Hz).[8] Although the experiment studied two specific positions of the epiglottis (horizontal and upright), it is hard to find an epiglottis in the horizontal position in a clinical situation. In general, studies on the effects that the epiglottis has on voice are rare, and the role of the epiglottis in acoustics and aerodynamics is not clear.[9] According to our clinical observations on the movement of the epiglottis during phonation, the epiglottis tends to rise up in the patient who has a background of vocal music.

The purpose of this research is to determine what effects that changing the angle of epiglottis (Aepi) has on the acoustic and aerodynamic parameters of phonation. It was hypothesized that increasing the epiglottal angle will significantly affect the acoustic and aerodynamic parameters by changing the structure of the laryngeal cavity. The canine larynx was chosen because of its similarity to human larynges.[10]

Accepted for publication February 9, 2018.

Conflict of interest: The authors have no funding, financial relationships, or conflicts of interest to disclose.

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Journal of Voice, Vol. 33, No. 5, pp. 627−633
0892-1997

https://doi.org/10.1016/j.jvoice.2018.02.007

METHODS

Data acquisition

Fourteen excised canine larynges were obtained from animals sacrificed at Xiamen University Zhongshan Hospital for surgical skills practice. Data collection was performed at the Key Laboratory of Underwater Acoustic Communication and Marine Information Technology of Xiamen University. The research was approved by the Xiamen University Institutional Review Board (Xiamen University Zhongshan Hospital Laboratory Animal Management Ethics Committee). Fresh larynges were collected immediately after excision and cleaned in saline solution. Before starting the experiment, all of the extrinsic laryngeal muscles and connective tissue were removed. The excised larynges were then mounted on a homemade experimental platform (Figure 1).

Considering that there is no widely used parameter for describing the Aepi so far, we defined that the extension of the petiolus epiglottidis and the vocal folds form the Aepi, as shown in Figure 2A. To determine the anatomic Aepi of each larynx, inverse-trigonometric functions were used to calculate it after the length of the epiglottis, L1, and the distance between the anterior commissure and the projection of the glottis on the tip of the epiglottis, L2, were measured with a vernier caliper. The related measurements and calculation are shown in Table 1.

In an anechoic room, the arytenoid muscles of the excised larynges were stabilized using two steel needles to maintain the anatomic position of each larynx and to adduct the vocal folds. The airflow was produced by an air compressor (Xiamen Taixing Electrical Co. Ltd., Xiamen, China) through an artificial lung (a homemade cylinder, 8 cm in radius and 16 cm in height) to

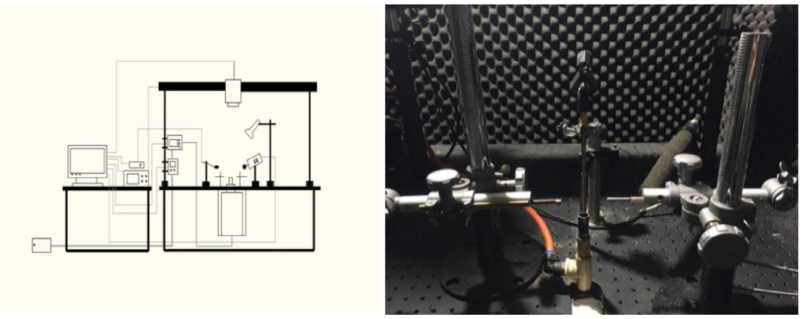
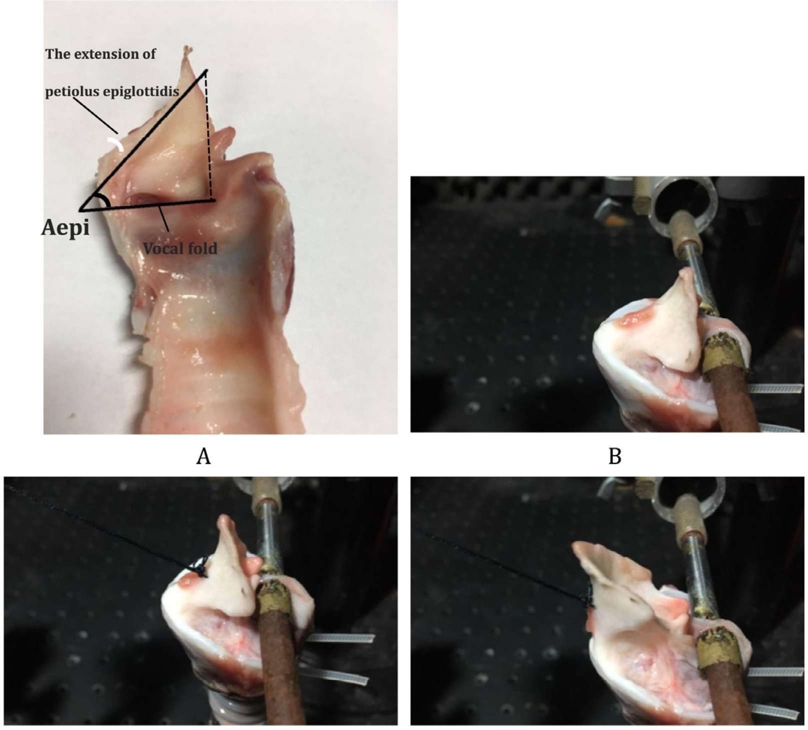

A
B

FIGURE 1. **A.** Sketch of the platform. **B.** Image of the platform.



A
B
C
D

FIGURE 2. **A.** The lateral view of the angle of epiglottis in an excised canine semilarynx. **B–D.** The superolateral view of control, 60° treatment, and 90° treatment of the Aepi, respectively, with a suture used to manipulate the Aepi.

TABLE 1.
Calculation to Determine the Angle of Epiglottis in 14 Excised Canine Larynges

Larynx	L1 (cm)	L2 (cm)	Aepi (°)
1	2.70	2.90	21.40
2	2.20	2.40	23.56
3	2.90	3.20	25.01
4	2.30	2.60	27.80
5	2.40	2.80	31.00
6	2.10	2.50	32.86
7	1.90	2.40	37.66
8	1.50	1.90	37.86
9	1.80	2.30	38.50
10	2.10	2.70	38.94
11	2.10	2.70	38.94
12	1.80	2.50	43.95
13	1.80	2.60	46.19
14	1.90	2.80	47.27

trigger the vibration of the vocal folds. MFR and onset phonation threshold flow (PTF) were measured with a digital airflow machine (MF-5706-N-10, Kongxin Instrument Co. Ltd., Nanning, China). A humidifier (022G877S, German Bairui Ltd., Starnberg, Barvarian State, Germany) was placed downstream of the artificial lung to avoid dryness on the surface of the laryngeal mucosa. This device has been used with success by Jiao et al.[11]

The acoustic and aerodynamic parameters of the control (unaltered larynges with anatomic Aepi) were measured first. The acoustic parameters included SPL, F0, jitter, shimmer, and frequency of formants 1−4 (F1′, F2′, F3′, and F4′). The labels, F1′−F4′, were used to indicate formants 1−4. Because there is no vocal tract in the present study, a different labeling convention from other experiments, which used an intact vocal tract, is used to differentiate the formant results calculated. The aerodynamic parameters included PTP, PTF, and MFR. To adjust the Aepi, a suture was tied to the anterior aspect of the epiglottis at the midline just inferior to the point where muscles attach. The tension of the suture line was adjusted to change the Aepi to 60° and 90° in all the 14 larynges (Figures 2A−C), and the acoustic and aerodynamic parameters were measured for these treatments as well.

Onset PTP was measured at the beginning of phonation using a pressure transducer (CWY100, Shanxi Chuangwei Ltd., Xi'an, China). When phonation was stable, the audio signals were recorded on *Cool Edit Pro* 2.1 software (Syntrillium Software Corporation, Phoenix, AZ) with a microphone (ECM-678, Sony Electronics Inc., Park Ridge, NJ) positioned at a distance of 15 cm from the vocal folds and at a 45-degree angle to the vocal folds. The SPL was measured with a sound level meter (WS1361, Shenzhen Wansheng Ltd., Shenzhen, China) placed 10 cm from the glottis. Trials were conducted at a sequence of at least 5 seconds of phonation followed by a 30-second break.

Data analysis

Each audio signal was cut to a 2-second audio clip by *Cool Edit Pro* 2.1 and was analyzed with *lingWAVES* software (WEVOSYS Co. Ltd., Forchheim, Barvarian State, Germany) to determine jitter, shimmer, and F0, and *Praat* software (version 5.3, http://www.praat.org) was used to determine F1′, F2′, F3′, and F4′. The anatomic Aepi of each larynx was calculated with inverse-trigonometric functions.

SPSS Statistics Version 23.0 (IBM Corp., Armonk, NY) was used to apply simple linear regression to determine the relationship between the anatomic Aepi and the acoustic and aerodynamic parameters of the 14 canine larynges. One-way analysis of variance (ANOVA) followed by Fisher least significant difference (LSD) test was utilized to analyze the differences in the acoustic and aerodynamic parameters among the control, 60° treatment, and 90° treatment. A significance level of $\alpha = 0.05$ was used.

RESULTS

The linear relationship between the anatomic Aepi and the acoustic and aerodynamic parameters

Figure 3 depicts the anatomic Aepi in 14 canine larynges in order of size. The mean Aepi of the control was 35.02° (range 21.40°−47.27°). Figure 4 shows the relationships between the anatomic Aepi and jitter, shimmer, F1′, and F2′ when the subglottal pressure (Ps) is 1.5 kPa. Figure 4A illustrates a negative relationship between the anatomic Aepi and jitter ($P = 0.005$, $r = -0.701$). Figure 4B shows a similar correlation of the anatomic Aepi and shimmer ($P = 0.018$, $r = -0.621$). The negative relationship between the anatomic Aepi and F1′ ($P = 0.013$, $r = -0.646$) and F2′ ($P = 0.038$, $r = -0.602$) is depicted in Figure 4C and 4D. All of the regression analyses fit a normal distribution and have homogeneity of variance of the residuals.

The variance of acoustic and aerodynamic parameters of different Aepi

The ANOVA results of the acoustic and aerodynamic parameters for all three treatments are shown in Table 2. Allowing for the heterogeneity of variance in F1′ and shimmer, there was no significant difference across the three treatments. However, there was an increasing trend in MFR and SPL as Aepi increased. Table 2 shows the results of Fisher LSD test with MFR and SPL. MFR in the 90° treatment was significantly greater than in the control ($P = 0.030$), but no significant

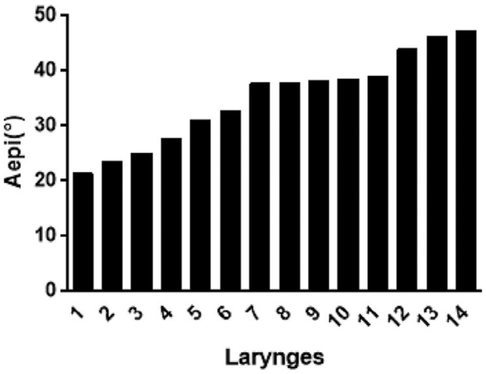


FIGURE 3. The anatomic angle of epiglottis in 14 canine larynges in order of size. The mean value was 35.02° (range 21.40°−47.27°).

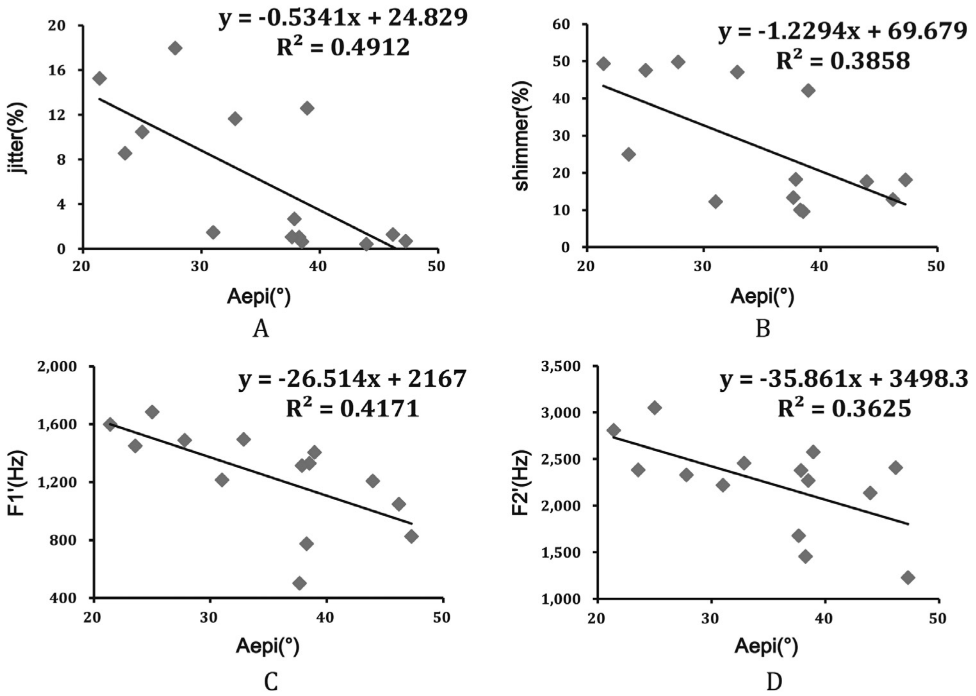


FIGURE 4. **A–D.** The simple linear regression of the anatomic Aepi of 14 canine larynges with jitter, shimmer, F1′, and F2′ when the Ps is 1.5 kPa.

difference was found between the control and the 60° treatment or the 60° treatment and the 90° treatment ($P = 0.275$ and $P = 0.258$, respectively). Similarly, SPL in the 90° treatment was significantly greater than in the control ($P = 0.033$), but no significant difference was found between the control and the 60° treatment or the 60° treatment and the 90° treatment ($P = 0.319$ and $P = 0.237$, respectively). From Figure 5A and 5B, we can see that MFR and SPL increase as Aepi increases. Even if there was a significant difference in shimmer across the three treatments ($P = 0.004$), the test of homogeneity of variance showed heteroscedasticity in jitter, shimmer, and F1′.

Onset PTP and PTF were collected in all the 14 specimens, and ANOVA results of all three treatments are shown in Table 3, and the results of the Fisher LSD tests are shown in Table 2. There was a significant difference in PTP across the three treatments ($P = 0.019$). PTP for the 90° treatment was significantly greater than for the control ($P = 0.021$), but no significant difference was found between the control and the 60° treatment or the 60° treatment and the 90° treatment ($P = 0.097$ and $P = 0.219$, respectively). PTF also had a significant difference across the three treatments ($P < 0.001$). Furthermore, significant differences were found between the control and the 60° treatment and

TABLE 2.
Fisher Least Significant Difference Test Results of MFR, SPL, PTP, and PTF in Different Treatments of 14 Canine Larynges

	Treatment		(I–J)	Standard Error	*P*
MFR	Control	60°	−0.2293	0.2072	0.275
	Control	90°	−0.4671	0.2072	0.030*
	60°	90°	−0.2379	0.2072	0.258
SPL	Control	60°	−1.7750	1.7601	0.319
	Control	90°	−3.8893	1.7601	0.033*
	60°	90°	−2.1143	1.7601	0.237
PTP	Control	60°	−0.0836	0.0492	0.097
	Control	90°	−0.1450	0.0492	0.005*
	60°	90°	−0.0873	0.0492	0.219
PTF	Control	60°	−0.1271	0.0449	0.007*
	Control	90°	−0.3336	0.0449	<0.001*
	60°	90°	−0.2064	0.0449	<0.001*

* $P < 0.05$

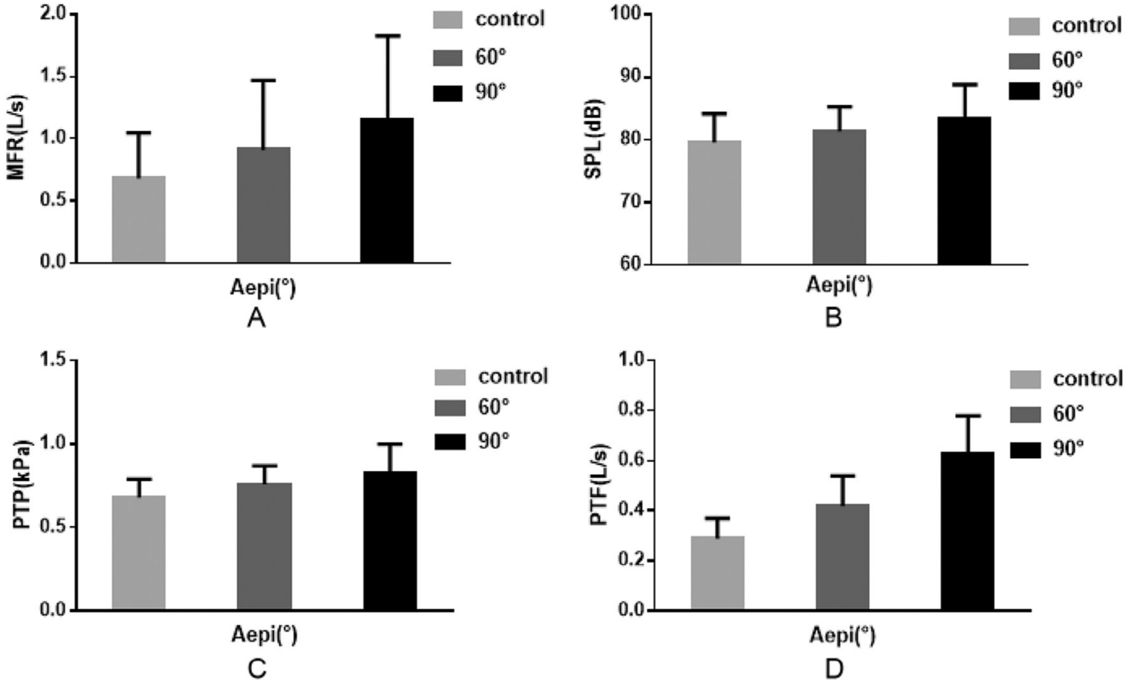


FIGURE 5. **A–D.** Comparisons of mean values of MFR, SPL, PTP, and PTF among different treatments in 14 canine larynges.

TABLE 3.
The Average Level ± Standard Deviation (SD) and ANOVA Results of Acoustic and Aerodynamic Parameters Under Different Treatments of 14 Canine Larynges When Ps Is 1.5 kPa

	Control	60°	90°		
	Mean ± SD	Mean ± SD	Mean ± SD	F	*P*
MFR (L/s)	0.68 ± 0.37	0.91 ± 0.56	1.15 ± 0.68	2.543	0.092
SPL (dB)	79.59 ± 4.62	81.36 ± 3.92	83.48 ± 5.33	1.571	0.100
F0 (Hz)	138.23 ± 40.82	136.45 ± 37.49	160.82 ± 43.13	1.571	0.221
Jitter (%)	6.13 ± 6.33	6.82 ± 6.76	3.71 ± 3.99	1.104	0.342
Shimmer (%)	26.63 ± 16.45	26.17 ± 14.79	11.21 ± 4.96	6.294	0.004
F1′ (Hz)	1238.54 ± 341.10	1227.78 ± 356.19	1007.01 ± 530.85	1.366	0.267
F2′ (Hz)	2242.46 ± 494.90	2304.33 ± 342.42	2316.55 ± 522.06	0.104	0.901
F3′ (Hz)	3370.18 ± 401.83	3472.27 ± 419.28	3387.53 ± 569.22	0.189	0.828
F4′ (Hz)	4574.47 ± 443.23	4628.04 ± 389.15	4340.40 ± 452.97	1.698	0.197
PTP (kPa)	0.68 ± 0.11	0.76 ± 0.11	0.83 ± 0.17	4.378	0.019*
PTF (L/s)	0.29 ± 0.08	0.42 ± 0.12	0.63 ± 0.15	28.155	<0.001*

* $P < 0.05$

the 60° treatment and the 90° treatment for PTF ($P = 0.007$ and $P < 0.001$, respectively), indicating that PTF increases as Aepi increases. Figure 5C and 5D illustrates the mean values of PTP and PTF. Figure 6 shows the spectrogram of one larynx with a Ps of 1.5 kPa when the Aepi was adjusted to 60°.

DISCUSSION

This study showed that the Aepi influences the acoustic and aerodynamic parameters of excised canine larynges. When Ps was controlled at 1.5 kPa and measurements were taken while the Aepi was the anatomic angles 60° and 90°, MFR was significantly larger in the 90° treatment. This result agreed with the previous research of Finnegan and Alipour. They showed that the vocal tract became more open as the epiglottis was adjusted from a horizontal (0°) position to an upright position (90°), which caused glottal airflow resistance to decrease and MFR to increase.[8] However, the designs of our current study are different from Finnegan and Alipour's study in some aspects. For instance, our study used three Aepi, including the anatomic Aepi as a control. Their study only had two treatments, and they did not include the anatomic Aepi. In a clinical setting, we have observed that the epiglottis would elevate and even lean back in some people when they need to increase the volume of their voice for activities such as singing. This means that the Aepi is increasing, and this kind of movement during phonation was confirmed by the current research. The Fisher LSD test found that SPL was significantly higher for the 90°

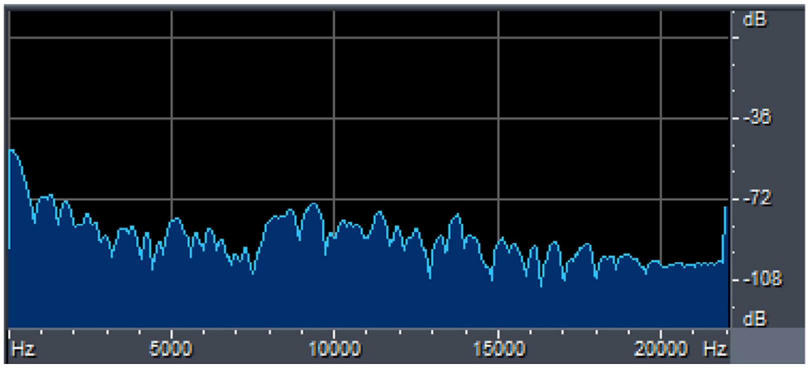


FIGURE 6. Spectrogram of one excised canine larynx with a Ps of 1.5 kPa when the Aepi was adjusted to 60°.

treatment than the control. A possible explanation is that when the Aepi increases, the vocal folds get tenser and this should theoretically cause F0 to increase. However, no significant difference in F0 was seen in the ANOVA of the three treatments. This can be explained as follows: (1) during observation of the specimens, it was noted that when the Aepi increases, not only does the tension of the vocal folds increase, but the length increases as well; and (2) the relatively high individual differences in the F0 of canine larynges resulted in the relatively high standard deviation of the F0, which was similar to what occurred in Alipour et al's experiment,[10] and this makes it difficult to determine differences with ANOVA.

Onset PTP and PTF are the minimum pressure and airflow required to begin phonation, respectively. Regner et al proposed that onset PTF could be a useful diagnostic parameter of the voice, particularly when used in conjunction with PTP to describe laryngeal resistance and aerodynamic power.[12] Our results supported those conclusions when we found that PTF was significantly different across all three treatments and that PTP was significantly different between the control and the 90° treatment in LSD test. Jiang et al presented that PTF is more sensitive to changes of vocal fold constituents, glottal shape, and vocal tract load than PTP.[9] According to the equation $U_{PTF} = L\sqrt{\frac{8X_0{}^3Bc}{T\rho}}$ (U_{PTF} is the phonation threshold flow, B is the damping coefficient, c is the mucosal wave velocity, L is the vocal fold length, X_0 is the neutral glottal width, T is the vocal fold thickness, and ρ is the glottal airflow density),[13] the reason for the increase in PTF is that when the Aepi increases, the vocal tract opens. This decreases the resistance and airflow density in the glottis, which allows PTF to increase. This study showed that PTF is more sensitive to vocal tract structure than both PTP and MFR.

The simple linear regression analysis of jitter and shimmer for the control showed a moderate negative correlation with Aepi. Jitter measures the degree of voice roughness, and shimmer measures the degree of voice hoarseness. However, jitter and shimmer are not widely used as a screening measure to differentiate between normal and abnormal voice because there is no direct correlation between jitter or shimmer and degree of voice impairment.[14] When a larynx has a relatively smaller Aepi, the vocal tract is more occluded and the glottal resistance is greater. This makes the airflow through the glottis more likely to be turbulent and interfere with the normal amplitude and frequency of the vocal signal during vocal fold vibration.

Formant refers to areas on a spectrum where energy is relatively concentrated. Formant is not only the determinant factor of sound quality but it also reflects the physical characteristics of a vocal tract resonator. It is believed that F1′ is related to the shape and resonance of the pharyngeal cavity and that F2′ has a connection with the shape and resonance of buccal cavity. According to the formula of formant frequency, $F_n = (2n-1)(c/4L)$ (F_n is the formant number; c is the velocity of sound, 340 m/s; and L is the vocal tract length, 17.5 cm in standard adult male),[15] it follows that when the Aepi increased, the vocal tract consisting of the epiglottis and aryepiglottic folds lengthened, increasing L and decreasing F_n. In simple linear regression analysis, F1′ and F2′ decreased as the individual anatomic Aepi increased in the control. This trend was consistent with the aforementioned formula and indicated that the change in Aepi has a significant effect on the formant frequency and timbre, and it may be related to the formation of different singing styles. However, there was no significant difference among the three treatments, although the frequency of F1′ in the 90° treatment decreased to about 1000 Hz. Therefore, the results indicate that the frequency of F1′ decreases when the Aepi increases as the vocal tract elongates, and the effect of the Aepi on F2′ remains to be further studied. Additionally, we reviewed previous studies and did not find any that focused on the formant of canine larynges. Furthermore, considering the formula of formant frequency, the formant will change when the length of the vocal tract changes by removing the oral and pharyngeal structures of the vocal tract from the larynx, but the formant remained within the normal range in the present study. We presume that when the vocal folds vibrate, sound waves propagate upward and downward simultaneously, and enter another tract consisting of the trachea and artificial lung under the glottis, creating the formants measured in our study. Future research should be done on this subglottic vocal tract.

The present study has several limitations. First, we did not separate the aryepiglottic folds from the epiglottis. When we adjusted the Aepi of larynx, not only was the position of the epiglottis changed, but also the position of the aryepiglottic folds. Future studies should focus on the aryepiglottic folds and epiglottis separately to specify the functions of these two supraglottic structures in phonation. Second, this experiment did not distinguish the shape of the epiglottis, which may have an effect on the shape of vocal tract and even on some parameters such as formant. Third, canines and humans walk in different modes, so the change of parameters in a canine may not occur in a human. Lastly, the structures that have been removed from the model in the present study likely affected the findings, and therefore, *in vivo* studies on epiglottis in humans and canine are needed in future.

CONCLUSIONS

The aim of this study was to explore the effects of the Aepi on the acoustic and aerodynamic parameters in excised canine larynges. The Aepi was proposed for the first time in this study as a more precise description of epiglottal position and to contribute to the understanding of the functions of supraglottic structures in phonation. Future efforts should address the effects of the Aepi on aerodynamic and acoustic parameters in humans, especially singers. In this study, it was discovered that (1) the Aepi plays an important role in the phonation and resonation of excised canine larynges; (2) larynges with larger Aepi have a more open vocal tract, which makes it difficult for turbulent flow to form, thus decreasing jitter and shimmer and increasing MFR and SPL; and (3) the vocal tract elongates when the Aepi increases, and this movement may have an effect on F1′ and F2′.

Acknowledgment

This work was supported by the National Natural Science Foundation of China (NSFC) 81371080.

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