Short Report: Acoustic Characteristics of Voiceless Bellowing Typical of Bovine Rabies

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Abstract. The use of auditory data visualization techniques was useful for distinguishing voiceless bellowls of a rabid cow from the bellowls of estrus-oriented cows, because rabid cows created two distinct sounds with a very short silence between inhalation and exhalation of the respiratory cycle. The auditory visualization techniques as a diagnostic tool may have the potential to serve as a basis for an Internet-based application for the early diagnosis of rabies.

Voiceless bellowing is a reliable clinical sign for distinguishing rabid cows from non-rabid cows, and it is used as a diagnostically useful sign. To date, most research has focused on distinguishing rabid cows by analyzing their bellowls through listening. The purpose of this report is to describe the practical application of auditory data visualization techniques to identify voiceless bellowls from estrus-oriented cows, despite the fact that only a few studies in this area have been reported.

A 1.5-year-old Hereford cow on a commercial farm (coordinates: 15°25′ S, 28°17′ E), which keeps 100 dairy cows in the Central Province of Zambia, was used as a rabid case in 1987. On April 9, 1987, the cow was bellowing and yawning for what seemed to be estrus reasons for several days. The cow had been frequently and aimlessly walking around, continuously salivating, tossing the head with abdominal pressure during exhalation, and also suffering from difficulties with swallowing water during respiration until the seventh day of the course (April 15), when the cow was slaughtered. This act was carried out because of the suspicion of rabies and to avoid consequent infection of other mammals in accordance with the official regulations of the Zambian government.

Confirmation of Negri bodies in the brain by pathological examination at a veterinarian laboratory provided a definitive diagnosis of rabies.

Three estrus Holstein–Friesian milking cows (aged 5–6 years old) on a dairy farm at the Hakkogakuen Hokkaido Agricultural Technical College were used as controls in 2010. This college is located in Sapporo (coordinates: 141°21′ E, 43°04′ N), Hokkaido, Japan, and it has approximately 40 Holstein–Friesian milking cows on its dairy farm and approximately 150 acres of land for education of college students on dairy cattle management practices.

On April 15, 1987, video and acoustic signals of a bellowing rabid cow were recorded on a videotape recorder (FujiFilm, Tokyo, Japan) at a distance of 2–10 m. The video and audio signals were subsequently transferred from magnetic tape to a DVD in 2009. Then, a stereo digital voice recorder (ICR-PS185RM [S]; Sanyo, Osaka, Japan) sampled the digital audio signals on the DVD at 44.1 kHz. Acoustic signals of the bellowing of the three estrus cows were recorded onto a stereo digital voice recorder during milking at a distance of around 2 m.

Noises in the audio signals from both the rabid cow and the controls were deleted using Audio Creator software (Cakewalk by Roland, Hamamatsu, Japan), and the recorded audio signals were analyzed using Praat software on a PC running Windows XP. Data for acoustic factors were read from the acoustic waves of the review and edit panel of Praat software, and they were analyzed by use of the built-in function of the confidence interval in Excel; a value of \( P < 0.05 \) was considered significant.

To distinguish the bellowing voices of the rabid cow, acoustic signals were recorded from the controls for comparative analysis (Table 1). The mean inhaling time and pitch of the rabid cow and controls during inhalation were 527 versus 334 ms and 362 versus 144 Hz with significance \((P < 0.05)\), respectively. During the exhaling phase, no differences in the acoustic signals were seen between the rabid cows and controls. In particular, it should be noted that mean silence time appeared for 41 ms during the transition from inhalation to exhalation, whereas no silence was seen in the controls.

We next compared typical examples of acoustic signals from the rabid cow and the controls (Figure 1). Comparison between the inhaling phases revealed that the rabid cow showed a pitch at the frequency of 250–450 Hz (Figure 1E–F), whereas the control did not. Comparison between the exhaling phases revealed that the rabid cow showed a higher pitch (Figure 1E–F) than the controls. Furthermore, the spectrograms of the rabid cow revealed silence between the inhaling and exhaling phases of respiration.

The rabid cow created a rather high bellowing, even during the inhaling phase, which is not expected to occur with normal bellowing. Thus, the vocal cords of the rabid cow likely vibrated irregularly because of the air flow from the nostrils into the lungs. This process resulted in silence between the inhaling and exhaling phases of respiration. These results also suggest that the rabid cow created two distinct voices during the inhaling and exhaling phases that were separated by silence or breadth between them; the auditory impression of this separation may have been regarded as voiceless bellowing.

Although voiceless bellowing was heard from the rabid cow in only 6 of 21 inhalations (Table 1), it could still be regarded as a unique clinical sign. However, the sample size needs to be increased from \( N = 1 \) to confirm that the sounds made by the rabid cow are typical of rabid cows in general.

The rabies virus spreads through the central nervous system, which is considered to be associated with vagus nerve paralysis, because the vagus nerve is responsible for reticulomorphinal contractions. It may contribute to a disturbance in swallowing water and reticulomorphinal contractions, resulting in ruminal distension. Thus, this distension may

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### Table 1
Comparisons of respective acoustic signals during inhalation and exhalation

<table>
<thead>
<tr>
<th>Animals</th>
<th>Voices heard</th>
<th>Inhaling time (ms)</th>
<th>Sound pressure level (dB)</th>
<th>Pitch of voice (Hz)</th>
<th>Pitch duration (ms)</th>
<th>During transition: silence (ms)</th>
<th>Exhaling time (ms)</th>
<th>Sound pressure level (dB)</th>
<th>Pitch of voice (Hz)</th>
<th>Pitch duration (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabid cow (N = 1)</td>
<td>21</td>
<td>527 (445–610; N = 21)</td>
<td>53 (51–55; N = 20)</td>
<td>362 (276–447; N = 6)</td>
<td>309 (153–465; N = 6)</td>
<td>41 (26–55; N = 6)</td>
<td>1.069 (930–1,209; N = 21)</td>
<td>62 (60–64; N = 21)</td>
<td>172 (168–177; N = 21)</td>
<td>815 (466–1,164; N = 21)</td>
</tr>
<tr>
<td>Controls (N = 3)</td>
<td>14</td>
<td>334 (230–438; N = 14)</td>
<td>54 (51–57; N = 12)</td>
<td>144 (35–252; N = 2)</td>
<td>86 (3.7–169; N = 2)</td>
<td>NA</td>
<td>1.360 (944–1,176; N = 14)</td>
<td>60 (57–63; N = 14)</td>
<td>173 (145–201; N = 14)</td>
<td>529 (297–761; N = 14)</td>
</tr>
</tbody>
</table>

P values: *P < 0.05*, ns (P > 0.05).

Confidence intervals are given by the confidence level of 95%. Numbers in each cell represent mean, 95% confidence interval, and number of samples. NA = not available; ns = not significant (P > 0.05).

**Figure 1.** Comparison of typical acoustic data between voices sampled from the rabid cow and the control. (A–F) The dashed vertical line of each panel separates the inhaling and exhaling phases of a respiration cycle. I and E denote inhaling time and exhaling time, respectively. F1 and F2 in G denote formants, and the arrow indicates a white blank on transition from inhaling to exhaling phases at the frequency of F1 and F2 to mean silence (S) for approximately 19 ms. The spectrogram of the control in H indicates broad undefined patch to mean noises.
forcibly but partially prevent respiration. Therefore, the cow has to inhale as much air as possible, as fast as possible to ensure that sufficient air is available for exhalation.

In conclusion, taken together, a rabid cow bellows higher than a non-rabid cow during inhalation, and there is a very short silence, referred to as a voiceless sound, that occurs between inhalation and exhalation. Although the findings of this acoustic study on bovine rabies should be confirmed in a greater number of cases, they have the potential to serve as a basis for an Internet-based application for the early diagnosis of rabies. Such an application could easily be deployed to mobile devices such as the iPhone.

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Note: Supplemental sound file is available at www.ajtmh.org.
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