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Association of Vocalizations and Swine Behavior during a Human Approach Test

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Cover Page Footnote
This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2011-68004-30336 from the USDA National Institute of Food and Agriculture.

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Summary and Implications
The objective of this study was to determine if the duration and total number of pig vocalizations when divided into low and high call categories was related to pig behaviors during a fear test. Twenty barrows and 20 gilts were tested over two consecutive weeks between 1300 and 1900 hours using a human approach test (HAT). Throughout the test, vocalizations and behavior were recorded. These results suggest that while high calls are typically the primary measure of stress vocalizations, low calls are also meaningful measures during the stressor of HAT.

Introduction
Swine vocalizations may provide information on affective states. Vocalizations are often reported as call frequency (Hz), total number and duration of calls. Previous studies have reported that increased total number of high calls (≥1000 Hz) may be an indicator of negative affective states. However, few studies have investigated if low calls reflect affective states. Therefore, the objective of this study was to determine if the duration and total number of pig vocalizations when divided into low and high call categories was related to pig behaviors during a fear test.

Materials and Methods
Experimental design: The protocol for this experiment was approved by the Iowa State University Institutional Animal Care and Use Committee. The experiment was conducted between February and March, 2013. A total of 40 Yorkshire barrows and gilts with a mean (±SD) age of 101 (±9) days, selected for high-RFI (n=20 barrows and n=20 gilts) were tested.

Animals and housing: This work was conducted at the Lauren Christian Swine Research Center at the Iowa State University Bilsland Memorial Farm, near Madrid, Iowa. Barrows and gilts were housed in mixed sex groups (15 to 16 pigs/pen) and each pen contained one Osborne single spaced electronic feeder (FIRE®, Osborne Industries, Inc., Osborne, KS) positioned at the front of the pen.

Fear test: All pigs were tested using a human approach test (HAT). Testing occurred over two consecutive weeks between 1300 and 1900 hours. The pigs were tested individually within a 4.9 x 2.4 m test arena. Arena sides were lined with black corrugated plastic at a height of 1.2 m. During testing, pigs were individually moved from their home pen to the test arena, which was located in a different room within the same building. Each individual pig was allowed to habituate for one minute in a weigh scale where it could not see the arena. At the conclusion of the one minute the weigh scale door was opened into the back corner of the test arena and an unfamiliar human wearing orange coveralls was standing still at the center of the opposite wall (Figure 1). Each pig was assessed for 10 minutes.

Figure 1. Arena where pigs received the human approach test.

Vocalization collections: Digital audio recordings of pig vocalizations during HAT were captured with a Marantz PMD 661 recorder (Marantz Corp., Kanagawa, Japan) and a Crown PZM185 microphone (Crown Int., Elkhart, IN). The recorder digitized the audio into a wav file at 16 bit and a sampling rate of 48 kHz. Raven software (Raven Pro 1.5, The Cornell Lab of Ornithology, Ithaca, NY) was used to produce spectrograms (Hanning window, window size of 1024 samples and overlap at 75%; time grid size of 256 samples; frequency grid size of 46.9 Hz) and manually identify vocalizations.

Vocalization measures: Two call categories were developed based on published literature: low defined as <1000 Hz and high defined as ≥1000 Hz. Within these call categories duration and total number of vocalizations were calculated. Duration was defined as the length of the vocalization which contained 90% of the energy. The total number of
vocalizations within the low and high call categories were counted for each pig.

**Behavioral collection:** Three color cameras (Panasonic, Model WV-CP-484, Matsushita Co. LTD., Kadoma, Japan) were placed above the test arena for video collection. Video was collected onto a computer using Handy AVI (HandyAVI version 4.3 D, Anderson’s AZcendant Software, Tempe, AZ, USA) at 10 frames/sec.

Continuous observation of video was done by one observer using Observer software (The Observer XT version 10.5, Noldus Information Technology, Wageningen, The Netherlands). Behaviors analyzed were touch, escape attempt, and freeze (Table 1).

Table 1. Definitions for collected behaviors

<table>
<thead>
<tr>
<th>Measure</th>
<th>Definition</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Touch</td>
<td>Total number of times touching the human during the human approach test and total length of time to first touch the human; touch was considered interaction of the mouth, nose, and/or face of the pig touching any part of the human</td>
<td>Frequency; Latency (s)</td>
</tr>
<tr>
<td>Escape attempt</td>
<td>Total number of times the pig had either both front hooves or all four hooves off the arena floor in an apparent attempt to remove itself from the test arena</td>
<td>Frequency</td>
</tr>
<tr>
<td>Freeze</td>
<td>Total number of times the pig did not move any portion of its body for ≥3 s</td>
<td>Frequency</td>
</tr>
</tbody>
</table>

**Data analysis:** Data were analyzed using Proc Corr to calculate spearman correlations using SAS (SAS Institute Inc., Cary, NC, USA). The significance level was fixed at $P \leq 0.05$ and tendency at $P \leq 0.10$.

**Results and Discussion**

**Low calls:** Low call duration was negatively correlated with the latency to first touch ($P = 0.03$). This may suggest that longer low calls may be weakly related to increased approach motivation. Total number of low calls were positively correlated to the number of escape attempts ($P = 0.05$); suggesting that increased number of low calls was weakly related to pig fearfulness during HAT. No other measures of low calls were related to other behaviors collected (Table 2).

**High calls:** Total number of high calls tended to be positively correlated to the frequency of touches and negatively correlated to the latency to first touch. This may suggest that pigs with more frequent high calls have higher motivation to approach an unfamiliar human. Total number was positively related to the frequency of escape attempts; suggesting that increased total number of high calls is weakly related to an increase in pig fearfulness during HAT. No other measures of high calls were related to other behaviors collected (Table 2). While high calls are typically the primary measure of stress vocalizations, these results may suggest that low calls are also meaningful measures during the stressor of HAT.

**Acknowledgements**

This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2011-68004-30336 from the USDA National Institute of Food and Agriculture.

Table 2. Relationship between behaviors and low and high vocalizations. (*) indicates significance and (**) indicates tendency.

<table>
<thead>
<tr>
<th></th>
<th>Low Call Duration</th>
<th>Total Number of Low Calls</th>
<th>High Call Duration</th>
<th>Total Number of High Calls</th>
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</thead>
<tbody>
<tr>
<td>Frequency of Touches</td>
<td>$r^2=0.09$</td>
<td>$r^2=0.02$</td>
<td>$r^2=-0.27$</td>
<td>$r^2=0.28$</td>
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<tr>
<td>$P=0.56$</td>
<td>$P=0.90$</td>
<td>$P=0.96$</td>
<td>$P=0.08$</td>
<td></td>
</tr>
<tr>
<td>Latency to First Touch</td>
<td>$r^2=-0.34$</td>
<td>$r^2=-0.08$</td>
<td>$r^2=-0.01$</td>
<td>$r^2=-0.27$</td>
</tr>
<tr>
<td>$P=0.03$</td>
<td>$P=0.62$</td>
<td>$P=0.96$</td>
<td>$P=0.09$</td>
<td></td>
</tr>
<tr>
<td>Frequency of Escape</td>
<td>$r^2=0.15$</td>
<td>$r^2=0.32$</td>
<td>$r^2=0.31$</td>
<td>$r^2=0.34$</td>
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<tr>
<td>$P=0.33$</td>
<td>$P=0.12$</td>
<td>$P=0.12$</td>
<td>$P=0.04$</td>
<td></td>
</tr>
<tr>
<td>Frequency of Freezes</td>
<td>$r^2=0.05$</td>
<td>$r^2=0.04$</td>
<td>$r^2=0.04$</td>
<td>$r^2=0.14$</td>
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<tr>
<td>$P=0.78$</td>
<td>$P=0.81$</td>
<td>$P=0.84$</td>
<td>$P=0.39$</td>
<td></td>
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