Hot-water spraying is a sensitive test for signs of life before dressing and scalding in pig abattoirs with carbon dioxide (CO₂) stunning

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This study investigated the benefits of hot-water spraying (HWS) as a diagnostic test to verify the absence of signs of life (SOL) before scalding in pigs slaughtered with carbon dioxide (CO₂) stunning. A total of 37 108 finishing pigs from five German abattoirs (A to E) operating at 55 to 571 pigs per hour were assessed. Suspended pigs were sprayed onto the muzzle, head and front legs (143 to 258 s post sticking for 4 to 10 s, 57°C to 72°C). Any active movements during HWS were rated as positive test outcomes. In comparison, SOL were considered to be absent if a subsequent manual examination was negative and no active movements were observed following HWS. The incidence of pigs with activity during hot-water spraying (PWA) was restricted to two abattoirs (B: 0.25%; D: 0.02%; A, C, E: 0.00%). PWA showed movements of facial muscles (88%), mouth opening (78%), righting reflex (63%), isolated leg movements (35%) and vocalization (4%). The manual examination was positive in 71% of PWA (corneal/dazzle reflex: 67%/53%, nasal septum pinch: 33%), whereas all inactive pigs tested negative (P < 0.001). The sensitivity for HWS as a test for SOL was calculated as 100%, dropping to 75% when only obvious and strong movements were taken into account. The specificity was >99.9% in either case. Any positive manual findings as well as any respiratory activity were instantly terminated using a penetrating captive bolt. Active movements triggered by the shot were shown to be an indicator for SOL (P < 0.001). Video analyses revealed that spontaneous movements (SM) following sticking were present in 100% of PWA as opposed to 3.1% in pigs without such activity (controls). Results for different categories of SM in PWA v. controls were as follows: 100% v. 2.6% for mouth opening, 16.0% v. 0.1% for righting reflex and 22.0% v. 0.9% for isolated leg movements (all P < 0.001). First mouth opening after sticking was observed later in PWA (28 ± 24 v. 10 ± 7 s), but mouth openings were observed for a longer period of time (141 ± 44 v. 27 ± 25 s) (both P < 0.001). PWA with shorter mouth-opening intervals showed higher movement intensities during HWS and more positive manual findings (P < 0.05). We conclude that HWS is a promising test for SOL. SM and sustained mouth opening in particular are indicators for compromised animal welfare and affected pigs should be shot by captive bolt.

Keywords: pigs, slaughter, carbon dioxide, hemorrhage, animal welfare

Implications
This study shows that current pig slaughter regimes involving carbon dioxide stunning carry a risk of conscious animals being exposed to painful further processing steps such as dressing and scalding. Business operators have a legal and social obligation to prevent this scenario. An automatic detection method would be preferable, considering the rarity of cases and the level of industry automation. We demonstrated that pigs with signs of life showed movements during automatic spraying with hot water. If combined with an automatic detection unit, the resulting system could provide a reliable last check at this stage of the slaughter process.

Introduction
Stunning with ≥80 vol% carbon dioxide (CO₂) in air is routinely used in commercial pig slaughter. Common exposure times induce a period of reversible unconsciousness and death is caused by a chest stick (Wotton and Gregory, 1986; Forslid, 1987; Martoft et al., 2003). Animal welfare is compromised when consciousness is present at any time in
the period between the end of the stunning intervention and death. Known risk factors include insufficient stunning effectiveness as well as delayed, ineffective or omitted 
sticking (Rodríguez et al., 2008; Von Wenzlawowicz et al., 2012; Llanch et al., 2013). The affected pigs run the risk of 
being exposed to further processing steps (usually scalding) if 
no additional stun/kill intervention is performed. Within the 
European Union, sustained unconsciousness and insensibility 
are statutory requirements and business operators are 
required to implement risk-based monitoring procedures. 
Further processing is only allowed once the absence of signs 
of life (SOL) has been verified (Council Regulation (EC) No 
1099/2009, 2009). For this purpose, hot-water spraying 
(HWS) has been suggested. The hypothesis is that following 
stunning and bleeding reactions to a painful stimulus can be 
triggered in pigs with SOL (Troeger and Meiler, 2006). The 
stimulus as well as the detection of triggered movements 
could be automated, preventing these pigs from being 
processed further. We, therefore, set out to (1) develop an 
automatic HWS device; (2) investigate the feasibility and 
performance of HWS as a diagnostic tool to verify that SOL 
are absent before further processing; (3) gain information 
about the SOL expressed at this stage of the slaughter 
process; and (4) identify indicators of sustained SOL. The 
detection system was developed simultaneously but is not 
part of this publication.

Animals, material and methods

Animals and abattoirs
A sample of 37 108 finishing pigs was assessed during 
routine slaughter in five commercial German abattoirs 
(A to E). Eligibility criteria included the use of CO₂ stunning, 
the business operator’s willingness to participate in the study 
and the accessibility of the bleeding line. Process 
parameters for stunning and sticking, as well as sample size 
distributions, are given in Table 1.

HWS
Before further processing, hot water was applied using a 
purpose-built spraying device (Figure 1). It consists of three 
nozzle holders, each equipped with up to three flat spray 
nozzles aligned in parallel within a rectangular trough 
(600 × 300 mm). The holders are connected to a distributor 
that is attached to a nearby line. An electric water 
heater is interconnected when necessary. Spraying is 
triggered when a programmable logic controller (LOGO! 
24RC, Siemens, Munich, Germany) receives information of a 
passing slaughter hook via a mechanical sensor (Schmersal, 
Wuppertal, Germany) and activates a solenoid valve 
(Buschjost, Bad Oeynhausen, Germany) located at the inflow 
of the system. Inflow volume can be adjusted using turncocks 
located at the inlet of the distributor as well as its outlets to 
the nozzle holders.

The system was centrally installed on the bleeding line, 
and the vertical water jets were adjusted to a height of 
~600 mm. Suspended pigs were, thus, sprayed onto the 
muzzle, head and front legs as well as other cranial body 
parts depending on the pigs’ size. HWS took place while the 
animals were being conveyed from sticking toward further 
processing. The stick-to-spray interval range was 143 
to 258 s, varying between abattoirs due to differences 
regarding the accessibility of the bleeding line. Within the 
same abattoir, stick-to-spray intervals varied according to the 
number of pigs buffered between sticking and HWS as well 
as stops of the slaughter line. Each pig was sprayed for a 
period of 4 to 10 s depending on the abattoirs’ line speed. 
Water temperature was repeatedly measured (Testo 946; 
Testo, Lenzkirch, Germany) at the level of the muzzle and 
ranged from 55°C to 72°C. During HWS, pigs were closely 
obscured for any activity. All pigs were videotaped from a 
fixed angle for retrospective analysis of relevant sequences 
(1440 × 1080 p; HDR-PJ260VE and HDR-CX570E; Sony, 
Tokyo, Japan). Intensity scores were assigned to any 
movements recorded, ranging from hardly visible (1/3) to 
obvious (2/3) to strong (3/3).

Manual examination and captive bolt application
Following HWS, pigs were manually examined. An LED 
Flashlight (5200 cd; Fenix™ E21, Fenixlight, Shenzhen, China) 
was pointed at one eye and approached until nearly touching 
the cornea. The size and any response of the pupil were 
assessed. A positive dazzle reflex was noted if this procedure led 
to the closing of the eye. Subsequently, the cornea of one eye 
was touched using the tip of pliers (see next test). A positive 
corneal reflex consisted of closing of the eye. Finally, modified 
bent-nose telephone pliers were inserted into the pigs’ nostrils 
and the nasal septum was pinched. A positive nasal septum 
pinch was noted if this manipulation led to a distinct movement. 
In total, 25 094 pigs were manually examined (Table 1), as 8040 
pigs from abattoir B and all pigs from abattoir E were examined 
only when activity during HWS was present. A penetrating 
captive bolt gun (Cosh Magnum.25 Euro Stunner with 
black cartridges, Accles & Shelvoke, Sutton Coldfield, UK) 
was applied to pigs with positive manual findings or 
active movements following HWS, after which the manual 
examination was repeated. In addition, 50 pigs from abattoir B 
without activity during HWS were shot as well, in order to 
compare reactions.

Data assignment
Pigs were assessed in groups of 50 to 200 consecutive 
animals. The first pig of each group was tagged using animal 
marking spray, and the group number was displayed to the 
cameras when it passed by. Unique identifiers were assigned 
to each pig consisting of the group number and number 
within the group obtained by mechanical counters. Any 
findings of the HWS and manual examination were dictated 
to digital voice recorders (ICD-BX112; Sony), stating the 
affected animal’s identifier and signalment. The corresponding pig on the film was identified using these data 
as well as the synchronized digital timestamps in the audio 
and video recordings.
Video analysis of the bleeding process

In addition to the HWS, the bleeding process was video monitored as well. Obtained footage from both recordings was used to analyze the occurrence of spontaneous movements (SM) between sticking and HWS in abattoir B. For this purpose, pigs with activity during hot-water spraying (PWA) were compared with 4498 pigs without such activity (controls). For both groups, the frequencies of SM in general as well as of the following subtypes were determined: mouth opening, righting reflex and isolated leg movements. The total number of mouth openings observed per pig was counted. In addition, the following time intervals were calculated from the video files for both PWA and controls with SM: stick to HWS, stick to first mouth opening and first to last mouth opening. Intervals in between mouth openings were assessed as well.

Statistical analysis

Data were processed using Microsoft Excel and StatSoft Statistica 12. Fisher’s exact test was used for 2 x 2 contingency tables. Diagnostic parameters for HWS were obtained as follows: pigs tested positive if any active movements occurred during HWS. By comparison, pigs were considered suitable for further processing if the manual examination was negative and no active movements were present following spraying. Metric data were non-normal according to an extended Shapiro–Wilk W test. Distributions were, therefore, accessed using Mann–Whitney’s U-test. Goodman–Kruskal’s gamma was used for correlations.

Results

Activity in hot water

Activity during HWS occurred in 0.14% (n = 51) of the pigs. It was only seen in abattoirs B (0.25%; n = 50) and D (0.02%; n = 1). PWA showed movements of the following categories: activity of the facial musculature (88%; n = 45), mouth opening (78%; n = 40), righting reflex (63%; n = 32) and isolated leg-kicking or paddling (35%; n = 18). High-frequency vocalization was noted in 4% (n = 2) of those pigs. Activities were shown in varying intensities and combinations (Figure 2). Movement intensity was rated 1/3 or 3/3 in 31% (n = 16), respectively, and 2/3 in 37% (n = 19).

Results of the manual examination and captive bolt application

PWA had positive manual findings in 71% (n = 36) of the cases, whereas all inactive pigs tested negative (P < 0.001). The corneal reflex was present in 67% (n = 34), and the dazzle reflex was positive in 53% (n = 27) of the pigs. Responses to the nasal septum pinch were noted in 33% (n = 17). One pig did not vocalize during HWS but did during the manual examination. All three tests were positive in 31% (n = 16), whereas 20% (n = 10) was positive in either two
movements. Active movements following captive bolt terminated any positive manual captive bolt gun was applied to 44 PWA and it instantly was omitted in the sole case of PWA in abattoir D. The could be triggered by quickly approaching their eyes. Sticking
PWA and controls. Mouth opening was seen more often and (all
mouth-opening intervals were observed earlier in controls
higher in pigs with larger mouth-opening counts and lower

Results of the video analysis
Following sticking and before HWS, SM were observed in all PWA. All PWA showed mouth opening, whereas other types of SM were only seen in a part of PWA (Table 3). In pigs lacking activity during HWS, the incidence of SM was significantly lower ($P < 0.001$). In 22.0% of PWA and 18.4% of controls with SM, the hollow knife was pulled out right after sticking (i.e. tonic-clonic spasms). Their occurrence was limited to PWA with positive manual findings ($P < 0.05$). Parameters obtained regarding the performance of HWS are shown in Table 2.

<table>
<thead>
<tr>
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<th>95% CI</th>
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<tbody>
<tr>
<td>Sensitivity (%)</td>
<td>100</td>
<td>92.05 to 100</td>
<td>75.56</td>
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HWS = hot-water spraying.
Pigs tested positive when active movements occurred during spraying. Signs of life were considered to be absent if the following criteria were met: no active movements and no corneal reflex, dazzle reflex or reaction to a nasal septum pinch following spraying.

Movement intensities were scored as follows: hardly visible (1/3); obvious (2/3); strong (3/3).

All movement intensities are regarded as a positive test outcome.

Only obvious and strong movement intensities are regarded as a positive test outcome.

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Table 3 Indicators for signs of life before further processing for pigs from abattoir B; pigs with activity during hot-water spraying are compared with pigs without such activity (controls)

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Group</th>
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<tbody>
<tr>
<td>Spontaneous movements</td>
<td>PWA (n = 50)</td>
</tr>
<tr>
<td>Mouth opening</td>
<td>100&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>Righting reflex</td>
<td>100&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>Isolated leg movements</td>
<td>16.0&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>PWA</td>
<td>20.0&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
</tr>
<tr>
<td>Metric data</td>
<td></td>
</tr>
<tr>
<td>Time intervals (s)</td>
<td></td>
</tr>
<tr>
<td>Stick to hot-water spraying</td>
<td>50 140&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>Stick to first mouth opening</td>
<td>50 28&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>Consecutive mouth openings</td>
<td>48 7&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>First to last mouth opening</td>
<td>50 141&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mouth openings (n)</td>
<td>50 17&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

PWA = pigs with activity during hot-water spraying.
<sup>A</sup> Values within a row with different superscripts differ significantly at $P < 0.001$ (Fisher’s exact test, Mann–Whitney’s U-test).
<sup>B</sup> 739 mouth-opening intervals (540 from PWA and 199 from controls); 1 to 26 intervals obtained per pig.

mouth-opening intervals (Table 4). When mouth openings were observed for a longer period of time, significantly more manual test types were positive, whereas movement intensities during HWS were not affected. Stick-to-HWS intervals and stick-to-first mouth-opening intervals did not affect the number of positive manual test types or movement intensities during HWS.
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Table 4 Correlation coefficients for variables in pigs with activity during hot-water spraying

<table>
<thead>
<tr>
<th>Variables</th>
<th>Movement intensity (1 to 3)¹</th>
<th>Manual tests positive (1 to 3)²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time intervals (s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stick to hot-water spraying</td>
<td>0.07</td>
<td>−0.05</td>
</tr>
<tr>
<td>Stick to first mouth opening</td>
<td>0.09</td>
<td>0.15</td>
</tr>
<tr>
<td>Consecutive mouth openings³</td>
<td>−0.34**</td>
<td>−0.32**</td>
</tr>
<tr>
<td>First to last mouth opening</td>
<td>0.09</td>
<td>0.25*</td>
</tr>
<tr>
<td>Mouth openings (n)</td>
<td>0.38**</td>
<td>0.23*</td>
</tr>
</tbody>
</table>

Movement intensities during hot-water spraying and the number of positive test categories in the subsequent manual examination are correlated with variables regarding the moment of spraying as well as mouth-opening movements. ¹Movement intensities were scored as follows: hardly visible (1/3); obvious (2/3); strong (3/3). ²Manual tests performed: dazzle reflex, corneal reflex and reaction to a nasal septum pinch. ³Medians of 1 to 26 intervals obtained per pig. *P < 0.05 or **P < 0.01.

Discussion

Defining SOL

European abattoir operators are obliged to verify the absence of SOL before further dressing and scalding, but no definition is given for this non-medical term (Council Regulation (EC) No 1099/2009, 2009). It could be inferred that the legislator wanted death to be present before pigs enter the scalding tank. This would require cardiac arrest and irreversible loss of brain activity to be proven, which to date is an unfeasible task in abattoir environments. Recent publications have focused on indicators of consciousness and sensibility (EFSA Panel on Animal Health and Welfare (AHAW), 2013; Verhoeven et al., 2015) without referring to the regulation’s wording. We started from the premise that pigs lack SOL if our selected indicators of consciousness as well as any active movements are absent.

Performance and appropriateness of HWS

Following this definition, HWS is a reliable diagnostic test for SOL, as activity during HWS was observed in all pigs with SOL. An automatic detection module should ideally be able to detect all movement intensities, as pigs with lower movement intensities are not subordinate from an animal welfare point of view. This is supported by the fact that movement intensities did not correlate with the number of positive test types in the manual examination. The low frequencies of SOL observed in this study underscore the need for an automated individual testing method, as the sample sizes used in practice (e.g. checks by the welfare officer) are unlikely to detect pigs with SOL before scalding. HWS as described in this study could be implemented in most abattoir environments without any major building alterations. The method has been shown to work in electrically stunned pigs as well (Arnold et al., 2014) and may be applicable for other species too.

From an ethical point of view, using a painful procedure (hot water) to detect pigs with SOL is not ideal. However, HWS is supposed to be applied at a time when death should have long occurred (Wotton and Gregory, 1986), and testing pain perception is an established tool during bleeding (AHAW, 2013). Systems predicting irreversible unconsciousness by analyzing the individual blood loss have been investigated (Troeger, 2011) and are sometimes implemented industrially. If validated under present abattoir conditions, they could provide an alternative or additional safety measure for abattoirs with individual blood collection. However, when knives are pulled out right after sticking, these systems cannot detect insufficient blood loss.

SOL and consciousness

Our findings suggest that consciousness of varying degrees was present in pigs with SOL. Welfare was seriously compromised at least in some of the cases as our observations of righting movements and vocalizations imply (Grandin, 2013). Further, indicators incompatible with brain stem death were present in the majority of cases (European Food Safety Authority (EFSA), 2004). On the other hand, the presence of consciousness could be disputed on the basis that movements following noxious stimulation can occur in an unconscious state (Antognini et al., 2005). This is in line with the fact that some pigs reacted to the hot water but were negative in the manual examination. It is also true that clinical indicators lack sufficient validation by electroencephalographic methods, which are considered to be the most reliable tools for accessing consciousness (Humane Slaughter Association, 2014; Verhoeven et al., 2015). It must be stressed, however, that even their data are open to interpretation, especially in transitional stages of consciousness (Alkire et al., 2008).

Therefore, we propose that the absence of SOL must include the cessation of any movements, as is German law. This gives pigs the benefit of doubt and is an unambiguous criterion for the stakeholders involved.

Risk factors of SOL before further processing

Abattoir B obviously stood out against the remainder of the plants, providing 50 out of 51 PWA discussed here. The underlying reasons were not investigated in detail. However, dwell times and CO₂ concentrations were relatively low in this abattoir, whereas stun-to-stick intervals were rather long. These variables are known to be of crucial importance in preventing SOL (Atkinson et al., 2012; Von Wenzlawowicz et al., 2012; Llonch et al., 2013). In addition, the stick-to-hot water interval was relatively short in this abattoir, possibly increasing the likelihood of SOL at the time of HWS.

The implications of using hollow knives in the same way as conventional knives (i.e. pulling out the knife right after sticking) warrant closer examination, given the high frequency of this technique in both PWA and controls with SM. The initial blood loss may have been reduced due to an early collapse of the sticking wound. Further, this procedure was frequently observed when pigs piled up at the sticking position, putting pressure on the employee in charge and
possibly prolonging stun-to-stick intervals. This is consistent with other studies that have investigated the influence of different sticking techniques in more detail (Anil et al., 2000; Schweiger et al., 2013).

The sole PWA of abattoir D was overlooked at the sticking carousel and lacked an incision at the time of HWS. A peculiarity in this abattoir was that the six-knife sticking carousel was operated by a single employee who had <10 s per pig for sticking, knife-pulling and surveillance combined. An automatic system for the detection of pigs lacking incisions exists (Lysk et al., 2010) but is not implemented in German abattoirs. We propose that the frequency of slaughtered pigs lacking sticking incisions should be routinely monitored by business operators.

**Indicators of SOL before further processing**

Stick-to-hot water intervals did not differ for the PWA and control groups, showing that the occurrence of PWA was not a mere function of time. Rather, the groups differed already shortly following sticking as indicated in the respective stick-to-first mouth-opening intervals. A possible explanation is that the mouth openings (MO) seen within the control group were primarily gasping motions signifying a dying brain stem (Blackmore and Newhook, 1981; EFSA, 2004). On the other hand, the MOs in PWA seem to be consistent with the recovery of regular breathing. According to our findings, differentiating between the two by mere observation is not possible at the beginning of bleeding because the intervals between MOs were similar for both groups. The difference only becomes apparent later, given the mean number of MOs in PWA, which were a multiple of those seen in controls. PWA with lower mouth-opening intervals and, therefore, higher mouth-opening counts were then more likely to show stronger movements during HWS and possess more positive manual test types. In consequence, we suggest that any form of repeated respiratory activity during bleeding is unacceptable, irrespective of its regularity. The differentiation between gagging, gasping and breathing is ambiguous (compare Verhoek et al., 2015) and of little practical value in plants with high-throughput rates and short per-animal observation times.

Although these findings are based on pigs from a sole abattoir, recent studies have also found MO to be the predominant indicator for impaired welfare of pigs during bleeding (Atkinson et al., 2012). To our understanding, this underscores the importance of skilled employees who not only monitor the stunning but also the bleeding effectiveness. Business operators should encourage additional stun/kill interventions when necessary and provide adequate tools. A high-energy captive bolt gun seems to be a reliable tool for euthanasia in the context discussed here (Woods, 2012). It should be preferred over electrical methods, as long as doubts regarding their effectiveness following CO₂ stunning have not been disproven (EFSA, 2004). According to our findings, undirected movements following a captive-bolt shot indicate poor welfare in affected pigs.

We conclude that current pig slaughter regimes involving CO₂ stunning carry a risk of conscious pigs being exposed to painful further processing steps. HWS as described in this study is a robust, sensitive and feasible method to detect pigs with SOL. In combination with a sensitive detection unit, the resulting system could provide a justifiable measure to prevent longer-lasting suffering as long as painless methods or irreversible stunning methods are not at hand. All pigs with SOL before further processing could have been identified by observation of the bleeding process. This underscores the importance of adequate human and technical resources at this stage of the slaughter process.

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