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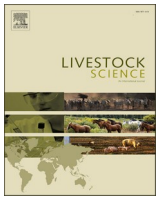

Exposure to natural stray currents of low voltage affects the behaviour and some stress biomarkers of weaned piglets

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HIGHLIGHTS

- Stray currents < 0.5V in the environment generate moderate behavioural and physiological stress responses in pigs and might affect their health and welfare.
- Pigs exposed to stray currents in the post-weaning unit spent less time in resting time and exhibiting socio-positive behaviours and, in the same time, pigs spent more time inactive in alert and exhibiting socio-negative behaviours.
- The blood oxidative status was increased in pigs exposed to stray currents.

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ABSTRACT

With the modernisation of pig breeding facilities, pigs may be exposed to both on-farm and off-farm sources of stray voltages. Data from ruminant species suggest that exposition to stray voltages may be a source of stress and impair animal welfare, but data are scarce for pigs. The aim of this study was to evaluate the impact of stray currents of voltages under 0.5 Volts in pig housing on piglet behaviours and some biomarkers after weaning. Two replicates of 820 piglets were reared in a farm naturally exposed to stray voltage for seven weeks. The difference in electrical potential between the floor and each drinker and feeder was measured every two weeks. Piglets exposed to high-voltage drinkers (HVD > 125 mV) spent more time orally manipulating pen mates ($P = 0.0031$). They also spent more time lying inactive with open eyes ($P = 0.0027$) and less time nosing pen mates ($P = 0.043$), but these effects were influenced by the voltage in feeders ($P = 0.0021$ and $P = 0.024$, respectively). Piglets exposed to high-voltage feeders (HVF > 50 mV) spent less time lying with their eyes closed ($P = 0.024$) and more time aggressing pen mates ($P = 0.0081$). Fifty days after entering the farm, blood hydroperoxide concentration was higher in piglets exposed to HVD ($P = 0.039$). The increase in socio-negative behaviours and oxidative stress in pigs exposed to stray voltages in pig housing suggested that stray voltages might have moderate detrimental consequences for piglets in post-weaning facilities.

1. Introduction

With the modernisation and intensification of modern pig breeding facilities, pigs may be exposed to many sources of stray currents. On-farm sources of stray are mainly induced by equipment (e.g. water pumps, lights, equipment for the automatic distribution of feed and the regulation of air flow or temperature in rooms). In animal housing, the neutral of the electrical system should be connected to the ground to ensure electrical safety. If grounding is deficient, animals may be exposed to stray voltage or contact currents. Ohm's law describes the

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relationship between voltage exposure and current conducted through an animal's body: the current intensity (measured in amperes) results from the voltage (measured in volts, V) divided by the resistance (in ohms) in the case of direct current or by the impedance in the case of alternating current. Contact voltage less than 10 V is a small stray current, measured between two points that can be contacted simultaneously by an animal (USDA-ARS Publication 696, 1991). Behavioural changes, their consequences and physiological impacts on welfare and production have been widely studied in dairy cows (Hillman et al., 2013; Rigalma et al., 2011, 2010; Wilson et al., 1996).

In swine, Gustafson et al. (1986) reported that the time spent drinking and the volume of water consumed was decreased in fattening pigs exposed to 3.6 V compared to those exposed to 2.8 V. Another study reported that a continuous or a transient source of stray voltage under 8V is unlikely to have detrimental consequences for fattening pigs (Robert et al., 1991). Production performances, particularly growth and feed conversion ratio, were not affected (Robert et al., 1991). Only subtle behavioural changes were noticed, such as an increase in time spent sitting and a decrease in time spent resting (Robert et al., 1991). The same team in another study reported no postural changes nor increase in aggressive behaviours, but they observed an increase in the time spent in front of the drinker when pigs where exposed to stray currents (Robert et al., 1992). Finally, they reported that the total body impedance of pigs decreased with age and body weight, therefore, for the same voltage, the current flowing through the body of pigs more than doubles from weaning age to slaughter (Robert et al., 1994). However, this scientific literature on contact voltage and its consequences in pigs is scarce and reports are outdated. All these studies were performed under laboratory-controlled conditions in pigs that were exposed to a 60 Hertz (Hz) alternating current sinusoidal voltage as provided by electricity suppliers in North America. In the field, higher frequency currents are common on farms. As described for cows, impedance of animals decreases as frequency of voltage increases, thus the animal receive higher amperage (energy) from higher frequency currents than what was studied in the literature (Hillman et al., 2013). Some field reports mentioned a reluctance by pigs to drink, reduction in appetite, restlessness, nervousness, increased aggressive behaviours, increased piglet crushing by their dam, impaired growth and an increased prevalence of health issues, such as porcine ear necrosis (Berton et al., 2018; Gillespie, 1984; Stetson et al., 1981; Wright et al., 1985). Altogether, these results suggest that stray current impair animal welfare, either via direct physiological effects, or because they are a source of stress for animals. Furthermore, since the 1980s, the equipment used in piggeries has changed considerably, and they now contain many more metal elements that are potential conductors of stray currents. For these reasons, the impact of stray currents on today's pig welfare must be re-evaluated.

The concept of farm animal welfare can, for practical purposes, be translated into the so-called Five Freedoms (freedom from thirst, hunger and malnutrition, freedom from discomfort, freedom from pain, injury and disease, freedom from fear and distress, freedom to express natural behaviour), which are the principles recognized by the World Organisation for Animal Health. However, this definition of animal welfare on farms is now outdated because this concept is much more complex than the Five Freedoms alone. In France, welfare is defined since 2018 as animal positive mental and physical state as related to the fulfilment of its physiological and behavioural needs in addition to its expectations (Anses, 2018). This state can vary based on the animal's perception of a given situation. In concrete terms, these measures vary depending on the age of the animals, but are based on the assessment of good body condition (such as body conformation, normal growth, absence of lesions), the absence of clinical signs of disease, and behavioural observations, as recommended in the Welfare Quality protocol (Dalmau et al., 2009). Appropriate behaviour can be assessed by looking at the frequency of expression of current social and non-social behaviours, estimated as positive or negative for the animal or its pen-mates, and the absence of expression of abnormal behaviours such as oral manipulation of pen-mates or stereotypies (for example, see Scollo et al., 2014). Although it would be needed for a complete evaluation of welfare (Reimert et al, 2023), so far, no easy tool has been developed to evaluate positive affective states on farm. Any event or chronic situation that degrades one of these five freedoms, or removes the possibility of feeling positive emotions, is a factor in the deterioration of welfare. Besides behavioural indicators and clinical observations, which are very time consuming when looking at large groups of animals, and may vary depending on the situation investigated, physiological measures are widely used to assess animal welfare (Godyń et al., 2019). The measure of cortisol in body fluids or hairs is used to assess hypothalamic-pituitary-adrenal (HPA) axis activity, as a non-specific revelatory of situations of stress (Casal et al., 2017; Merlot et al., 2011). Health problems can be also detected by the use of non-specific indicators of inflammation such as haptoglobin, whose levels also vary in response to stressful situations such as a change in feeding and housing conditions on pigs' stress (Pastorelli et al., 2012; Saco et al., 2010), or indicators of oxidative status such as blood antioxidant potential (BAP) and hydroperoxides (HPO) concentrations in the blood (Durand et al., 2022).

The aim of our observational study was to assess the effects of exposure to natural contact voltage below 0.5 Volts on a set of indicators of health and welfare, including behaviour, hair and saliva cortisol concentrations, and, haptoglobin, BAP and HPO concentrations in blood in weaned pigs. To our knowledge, this is the first field report about the effect of stray current on the welfare of piglets, and the first to evaluate the effects of stray currents in pig housing on pig physiology through biomarkers.

2. Materials and methods

2.1. Ethical statement

All procedures were approved by the ethical committee for veterinary research of ONIRIS (Nantes Atlantic National College of Veterinary Medicine, Food Sciences and Engineering, Nantes, France) (N°CERVO-2021-17-V) in May 2021 before the study started. All biological samples were collected by a veterinarian.

2.2. Animals and housing

This observational study was held in a wean-to-finish farm located in France. Two consecutive batches of 820 piglets (Large White x Landrace x Tai Zumu x Pietrain) were enrolled in the study (2 replicates, May-June and August-September 2021, 1640 piglets in total). Piglets were born in a farrowing unit, weaned at 28 days of age on average and moved to the wean-to-finish unit 4 to 5 days later. After birth, all tails were docked and males were surgically castrated after the injection of 0.5 mL of lidocaine in each testis and 0.4 mg per kg of body weight once of meloxicam intramuscularly. The farm was free of Porcine Respiratory and Reproductive Virus.

Arrival at the wean-to-finish unit was defined as day 0 (D0) of the trial. Piglets were vaccinated against *Mycoplasma hyopneumoniae* and porcine circovirus type 2 (PCV-2) on day 0. Piglets were divided between two identical rooms, with 12 pens per room and 34 ± 1 piglets per pen (4.0 × 3.5 m; length x width). Piglets were ear-tagged and allocated to the pens with their littermates and mixed with litters of sows of identical parity. At this stage, the investigators were blind to the presence and the level of stray voltage in pens, so the distribution of piglets in pens was random. The floor of the pens was fully-slatted. Pigs were fed *ad libitum* with a standard commercial pelleted dry feed distributed once a day in one stainless steel dry feeder (4 cm per pig of feeder linear space). During the first ten days, the piglets were fed with a first stage feed containing 18.5 % crude protein and 9.85 megajoule of net energy per kilogramme of feed (MJ NE/kg), and then, until the end of the post-

weaning period, they were fed with a second stage feed containing 18 % crude protein and 9.70 MJ NE/kg until the end of the study. Pigs had *ad libitum* access to water via two stainless steel drinkers delivering between one and two litres per minute. Finally, pigs had access to two enrichment objects (one metal chain and one wood block suspended on a metal chain). Light was on from 8:00 h to 19:00 h. Room temperature was 28 °C on day 0 and decreased from 0.5 degree every week until 24.5 °C at the end of the study on D50, when pigs were approximately 82 days-old.

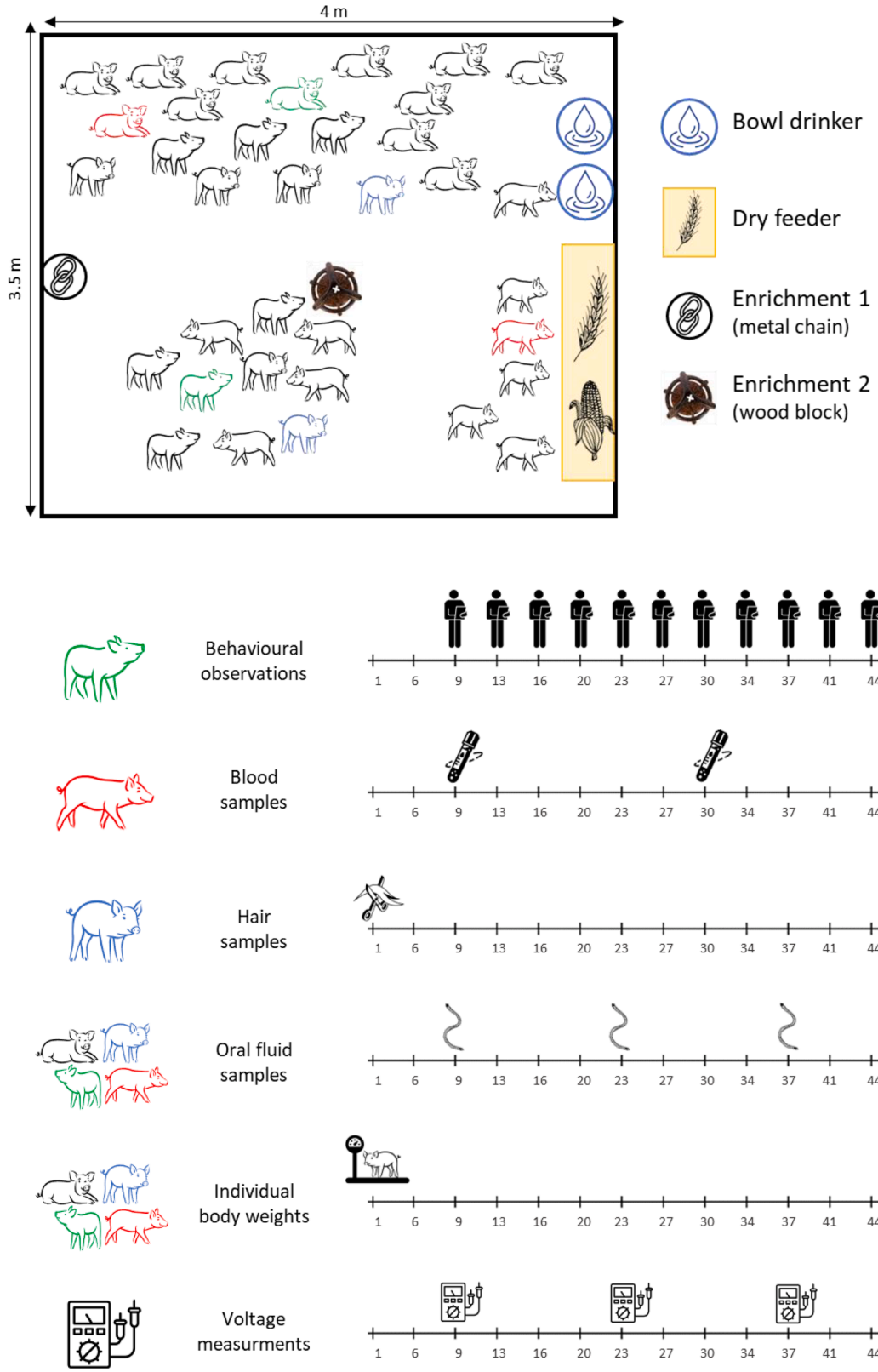

Fig. 1. Experimental design.

2.3. Experimental design

Weaners were exposed to a natural uncontrolled electric stray current of low voltage (< 0.5 Volt), previously evidenced in this farm unit. During the trial, stray voltage in each pen was measured at the level of each drinker and the feeder with a voltmeter (Multimeter C.A 5273 Portable 1000 V c.a. 10A c.a., Chauvin Arnoux, Asnières-sur-Seine, France) every two weeks (on day 9, day 23, day 37 and day 50) (Fig. 1). To measure voltages, one probe was placed in contact with the floor and the other probe in contact with the bottom of the feeder in an area without feed or with the drinker in a dry area, respectively. The recorded stray voltages ranged from 20 mV to 290 mV in drinkers and from 20 mV to 180 mV in feeders. In order to analyse the effect of stray voltage, pigs subsequently assigned to four groups depending on the mean level of contact voltages measured in their drinkers and feeders, calculated as the mean of the measures at the four time-points during the experiment. Voltage thresholds were chosen to be as close as possible to the median voltages measured during the trial. Median voltage measured in drinkers was 123.1 ± 57.4 mV (min-max= 10-290 mV) and median voltage measured in feeders was 49.7 ± 33.3 mV (min-max= 5-250 mV). Drinkers with a mean contact voltage above 125 mV were labelled high-voltage drinkers (HVD), while drinkers with a mean contact voltage under 125 mV were labelled low-voltage drinkers (LVD). Similarly, feeders with mean contact voltage over 50 mV were considered high-voltage feeders (HVF), while feeders with a mean contact voltage under 50 mV were considered low-voltage feeders (LVF). Each pig had a constant assignment in one group during the experiment. Two focal pigs were selected in each pen for behavioural observations, two others for blood samplings and two others for hair clippings (Fig. 1).

2.4. Measurements

2.4.1. Housing conditions and mycotoxin feed contamination

Temperature and humidity in rooms were continuously recorded using a data logger (Tinytag Plus 2 TGP-4500, Gemini Data Loggers, Chichester, United Kingdom). Feed samples were collected at each delivery and checked for mycotoxin contamination using a liquid chromatography-electrospray ionization-tandem mass spectrometry technique (Labocea, Ploufragan, France).

2.4.2. Pigs body weight

All piglets in both replicates were individually weight on D0 and D50 of the study.

2.4.3. Behavioural observations

At inclusion, two focal pigs were randomly selected per pen and identified with an extra ear-tag, and colour paint spray on their back (n = 96 piglets in total). One single observer was involved in behavioural observations (the first author) all along the trial. After an 8-day period of habituation to the presence of the observer, behaviours were scored twice a week (Tuesday and Friday) for 6 weeks (from day 9 to 48, Fig. 1), one hour in the morning (between 09.00 h and 11.00 h) and one hour in the afternoon (between 15.00 h and 17.00 h). The behaviour of each pig successively was recorded every 5 minutes (scan sampling method - (Altmann, 1974)), resulting in 24 scans per pig per day or 288 scans per pig in total (Fig. 1). For each pen and each day of observation, the occurrence of a given behaviour was expressed as a proportion, e.g. the number of scans when the behaviour was observed divided by the total scan numbers performed for the pen on that day (scans from the 2 focal pigs were pooled). The ethogram used in this experiment is presented in Table 1.

Table 1
Ethogram used in this experiment.

Behaviours	Observation	Description
Inactive behaviours	Standing inactive	Standing on all four legs without performing any activity
	Lying inactive with eyes opened	Ventral or lateral recumbency with eyes open without performing any activity
	Lying inactive with eyes closed	Ventral or lateral recumbency with eyes closed and head resting on the floor
Social behaviours	Nosing pen mate while standing	Touching, gently rubbing, nibbling or licking the body of a pen mate with the snout while standing on all four legs
	Nosing pen mate while lying	Touching, gently rubbing, nibbling or licking the body of a pen mate with the snout while lying down
	Aggressing pen mate	Head or shoulder knock, aggressively biting at any part of the body of a pen mate
	Manipulating orally pen mate	Biting, sucking, chewing or nibbling the tail, ear or any part of the body of a pen mates.
Exploratory behaviours	Manipulating orally enrichment objects	Chewing, nosing, sniffing, touching or rooting on objects in the pen (chain or wooden toy) with the snout
	Standing pen surface exploration	Nosing, sniffing, touching or rooting on the floor or the pen fixtures with the snout while standing, scraping the floor with the leg
	Lying pen surface exploration	Nosing, sniffing, touching or rooting on the floor or the pen fixtures with the snout while lying down
Locomotion behaviours	Walking	Walking in the pen
Maintenance behaviours	Eating	Eating from the feeder or nosing, sniffing, touching, rooting the feeder with the snout
	Drinking	Drinking water from drinker or nosing, sniffing, touching, rooting the drinker with the snout
	Eliminating	Defecating or urinating

2.4.4. Physiological parameters

2.4.4.1. Blood collection and analysis. Two piglets per pen were randomly selected from each batch and each room on D1 (84 piglets in total). They were identified with an extra ear-tag. Blood samples were performed on day 9, day 30 and day 50 (Fig. 1), after behavioural observations for D9 and D30. Blood samples were collected from pigs restrained with a snare by venepuncture from the jugular vein in 5 mL Vacutest® tubes containing heparin. One sterile needle per pig was used. Blood samples were immediately stored on ice and centrifugated at room temperature within four hours of collection at 3000g for 15 min. Plasma was stored at -20 °C until analyses.

2.4.4.2. Haptoglobin. Plasma concentration of haptoglobin was determined using a colorimetric method (Phase Haptoglobin Assay T801; Tridelta Ltd, Maynooth, Ireland) with an analyser (Konelab 20XT Multichannel Analyzer; Thermo Scientific, Courtaboeuf, France). The within-assay variation ranged between 0.09 % and 1.36 %, and the between-assay variation ranged between 7 and 11 %. Sensitivity was determined as 0.05 mg/mL of haptoglobin.

2.4.4.3. Blood indicators of oxidative status. HPO and BAP were chosen as plasma indicators of oxidative status and biomarkers of health in pigs as previously described by Buchet et al. (2017). HPO and BAP of heparin plasma were analysed using commercial kits (dROM and BAP tests, Diacron, Grosseto, Italy) on an automated analyser (Konelab 20, Thermo Fisher Scientific, Hampshire, UK). The dROM test measures the concentration of HPO generated by the peroxidation of lipids, proteins or nucleic acids (Alberti et al., 2000). Briefly, a 2 µL heparin plasma sample was added to 100 µL of acidic buffer and 1 µL of chromogen. Absorbance

was read every 98 seconds for 980 seconds at 505 nm. A standard curve with 8 points and internal control sample were used (intra and inter-assay coefficients of variation (CV) of 6 and 8 % respectively). The results of the test are expressed in CARRU (Carratelli Unit, 1 CARRU= 0.08 mg H_2O_2/100mL of sample). BAP results from the combined effects of many antioxidants such as uric acid, ascorbic acid, proteins, alpha-tocopherol or bilirubin (Benzie and Strain, 1996). Briefly, a 5 µL heparin plasma sample was added to a 210 µL solution of ferric chloride and thiocyanate derivate. Absorbance was read at 505 nm. A standard curve with 8 points and internal control sample were used (intra and interassay CV of 2 and 6 %, respectively). The results are expressed in µmol/L of equivalent vitamin C used as an iron-reducing reference agent. An Oxidative Stress Index (OSI) was calculated as the ratio of HPO to BAP (CARRU/µmol/L of Vit C) (Sharma et al., 1999).

2.4.4.4. Hair and salivary cortisol analyses. Hair samples were collected on D1 and D50 from another convenient sample of two piglets per pen in each batch (n = 80 in total) identified with an extra ear tag (Fig. 1). Pigs were clipped during restraint provided at weighing times. Hair samples were obtained from the dorso-lumbar area by shaving close to the skin with electric hair clippers, trying not to remove the root of the hair, and avoiding inclusion of the hair follicle in the sample. Hair samples were stored at room temperature in hermetically sealed bags and then frozen at -20 °C until analysis.

Cortisol was extracted from hair samples. First, approximately 150 mg of hair were washed three times in 3 mL of 99.5 % isopropanol for 3 minutes to eliminate contaminants that might interfere with the determination (in case of smaller samples the volume of isopropanol was reduced in order to maintain the same proportion). The hair samples were then allowed to dry 48 h in an airflow hood. The following day, samples were finely chopped using surgical scissors until hair segments were 0.3 cm maximum length. Each sample was then transferred to a 2 mL tube compatible with the grinder (Precellys, Bertin Technologies, Montigny-le-Bretonneux, France) and fitted with three metal balls. The tubes were run through the mill for 2 × 50 seconds at 4500 rpm, decanted in a microcentrifuge, then run through the mill again for 2 × 50 seconds at 4500 rpm. For cortisol extraction, 1 mL of 99.5 % methanol was added to approximately 50 mg of finely chopped hair, and the mixture was incubated at ambient temperature for 24 h with slow rotation. Then, the sample was spun in a microcentrifuge for 3 min at 12,000 rpm. Finally, 0.6 mL of the supernatants were dried using a vacuum centrifuge and stored at -20 °C. The dry extract was reconstituted in 400 µL phosphate buffer solution.

Pooled oral fluid was collected from each pen every two weeks (on day 9, day 23, day 37 and day 50) (Fig. 1) by suspending two untreated cotton ropes per pen at pig shoulder height for 30 minutes. Oral fluid samples were collected in the morning (between 9:00 and 11:00 am). The wet portion of the rope was manually wrung out inside a plastic bag to release the oral fluid. The oral fluids were stored in a 10 mL tube on ice and centrifuged at room temperature within four hours of collection at 3000g for 30 minutes. The supernatant was stored at -20 °C until analysis.

Concentrations of cortisol (in hair and in saliva) were measured in samples using a commercial kit (Cortisol Saliva Luminescence Immunoassay (RE62111/RE62119), IBL International, Hamburg, Germany). The assay was performed according to the instructions provided by the manufacturer. The limit of detection was 0.012 µg/dl.

2.5. Statistical analysis

Data management was performed with Excel 2019 (Microsoft Corporation, Redmond, USA) and statistical analyses were conducted using R Studio (R Core Team, 2020). For each analysis, the upper limit for the statistically significant effect was set at $P \leq 0.05$.

2.5.1. Performance data

Pigs nested within the level of contact voltage in drinkers and in feeders (four groups: LVD/LVF, LVD/HVF, HVD/LVF and HVD/HVF) was the experimental unit. Individual average daily weight gains were calculated and compared using a non-parametric Kruskal-Wallis test since the data were not normally distributed. Wilcoxon test was used post hoc to determine pair-wise differences between groups.

2.5.2. Behavioural data

Pen nested within the level of contact voltage in drinkers and in feeders was the experimental unit. Behaviours described as 'nosing pen mate while lying down' and 'nosing pen mate while standing' were ranged under 'nosing pen mates'. Similarly, 'standing pen surface exploration' and 'lying pen surface exploration' were ranged under 'Exploring the pen'. Behavioural time budgets are presented as the mean of the proportion of total scans, calculated from results obtained from each observation of each group, and the standard error of the mean (SEM). Mean behaviour relative proportions and SEMs were presented per week in graphs (see supplementary materials). Normality of residuals was tested using the Shapiro-Wilk test. Nosing pen mates, aggressing pen mates and manipulating pen mates were square root transformed. The best covariance structure was chosen, using the structure minimizing the absolute value of the Akaike information criteria (AIC). No significant week effect was detected. A mixed linear model was therefore applied to analyse effects of voltage levels in drinkers, feeders and their interactions with pen and batch as random effects only. Tukey's test was used as a post hoc to determine pair-wise differences between groups.

2.5.3. Biomarker data

Pigs nested within the level of contact voltage in drinkers and in feeders was the experimental unit, excepted for salivary cortisol (the pen was the experimental unit). Salivary and hair cortisol concentrations, plasma haptoglobin, BAP, HPO and OSI were presented as averages and standard errors, calculated from results obtained in each sampling time of each group. Biomarker data were logarithmically transformed if the model residuals were not normally distributed. A significant time effect was detected, therefore linear mixed models were applied to analyse data with voltage in drinker, voltage in feeder, their interaction and time as fixed effects and pigs, pens and replicate as random effects, except for salivary cortisol, for which random effects did not include the pig, but only the pen and the replicate. The best covariance structure was chosen, using the structure minimizing the absolute value of the AIC.

3. Results

3.1. Housing conditions and mycotoxin feed contamination during the trial

In both rooms, housing temperatures and relative humidity were stable throughout the trial. In the first batch, room temperatures were 26.0 ± 0.3 °C and 26.4 ± 0.8 °C, and percentages of relative humidity (% RH) were 55.2 ± 5.3 % RH and 56.4 ± 6.4 % RH in room 1 and 2, respectively. In the second batch, room temperatures were 27.0 ± 0.8 °C and 27.4 ± 0.9 °C, and percentages of relative humidity (%RH) were 64.2 ± 4.5 % RH and 63.8 ± 4.2 % RH in room 1 and 2, respectively. No mycotoxin contamination of feed was detected during the trial.

3.2. Performance data

At inclusion, average body weight was 8.7 ± 1.8 for the LVD/LVF group (n=424), 8.5 ± 1.9 for the LVD/HVF group (n = 285), 8.7 ± 1.8 for the HVD/LVF group (n = 446) and 8.7 ± 1.7 for the HVD/HVF group (n = 441). No significant difference in body weights at inclusion between the four groups was observed (P= 0.54). Average daily weight gain during the trial was 538 ± 92 for the LVD/LVF group (n = 320),

516 ± 92 for the LVD/HVF group (n = 284), 543 ± 95 for the HVD/LVF group (n = 446) and finally, 542 ± 92 for the HVD/HVF group (n=320). No significant difference between the four groups was observed (P= 0.67).

3.3. Behaviour

At the end of the trial, behavioural data from 13 HVD/HVF pens (25 HVD/HVF focal pigs), 14 HVD/LVF pens (26 HVD/LVF focal pigs), 8 LVD/HVF pens (16 LVD/HVF focal pigs) and 13 LVD/LVF pens (25 LVD/LVF focal pigs) were recorded. The four other focal pigs were excluded because of concomitant diseases (two presented arthritis or two died). Inactivity and oral manipulation of pen mates increased over time during the trial. Nosing peaked around the third week after weaning and pen mate aggression stayed stable (see supplementary materials).

Overall, time spent inactive (while standing or lying) represented 53 % of the total observation time (Table 2), divided into standing or lying inactive with eyes open (34 %) and lying inactive with eyes closed (13 %). HVF pigs spent less time lying with eyes closed than LVF pigs (P= 0.024). HVD pigs spent more time lying inactive with eyes open than LVD pigs (P= 0.0027).

Social behaviours represented 16.9 % of total observation time, divided into nosing (13.7 %), oral manipulation of pen mates (2.1 %) and aggression toward pen mates (1.1 %). Pigs exposed to HVD spent more time manipulating pen mates than those exposed to LVD (P= 0.0031). Pigs exposed to HVF spent more time aggressing pen mates than those exposed to LVF (P= 0.0081). Among pigs exposed to LVD, pigs exposed to HVF spent significantly more time aggressing pen mates (P= 0.020), but no statistical difference was observed among pigs exposed to HVD. Concerning the time spent nosing pen-mates, voltage in the feeder and in the drinker seemed to have opposite and interacting effects (interaction: P = 0.024), resulting in unclear differences between groups.

Non-social exploratory behaviours represented 15 % of total observation time, divided into pen exploration (12.2 %) and oral manipulation of enrichment objects (steel chain and wooden toy) (2.8 %). The proportion of time spent exploring the pen was affected by the drinkers x feeders interaction (P= 0.036). When the voltage was low in the feeder, pigs exposed to HVD spent less time exploring the pen than those exposed to LVD (P = 0.042). Finally, time spent manipulating the enrichments, walking or exhibiting ingestion-related behaviours (eating, drinking, eliminating urine and faeces) were not affected by either drinker voltage, nor feeder voltage, nor their interaction.

3.4. Cortisol

Salivary cortisol concentration from 13 HVD/HVF pens, 14 HVD/LVF pens, 8 LVD/HVF pens and 13 LVD/LVF pens were measured. At the pen level, salivary cortisol concentration increased over time during the trial (Fig. 2). Hair cortisol concentration on both D1 and D50 of the study from 20 HVD/HVF pigs, 20 HVD/LVF pigs, 11 LVD/HVF pigs and 17 LVD/LVF pigs were recorded. No significant differences were observed in salivary and hair cortisol concentrations between groups (P = 0.34 and 0.19) (Table 3).

3.5. Blood parameters

During the trial, 84 piglets were sampled on the three sampling occasions: 19 HVD/HVF pigs, 26 HVD/LVF pigs, 15 LVD/HVF pigs and 24 LVD/LVF pigs respectively. Whatever the day of the study, haptoglobin, and BAP were not influenced by contact voltages (Table 3 and Fig. 3). HPO concentration was significantly higher in the HVD/HVF group, which is supposed to be the more exposed to stray currents, compared with the three other groups (P= 0.027). In all groups, plasma HPO remained stable between day 9 and day 30 and increased significantly at day 50 (P= 0.0088).

4. Discussion

The aim of this field observational experiment was to study the effects of environmental stray currents of low voltage on the welfare of piglets in post-weaning units, analysing their behaviour and some health-related physiological biomarkers. The presence of stray currents under 0.5 Volts in the environment influenced piglet behavioural time-budget and increased the blood concentration in HPO, evocative of a mild oxidative stress. These observations might be indicative of an environmental stressful situation induced by the presence of stray voltages in housings.

One major limitation of our study is the lack of continuous recording of stray voltage and frequency during the experiment. We measured stray voltages in feeders and drinkers at only four time-points. This limitation makes our assignment of pigs to voltage groups debatable. Indeed major voltage variations could occur daily in a farm (Stetzer et al., 2016), and there are several on-farm contributions to step

Table 2
Behavioural activity of piglets during the trial (D9-D48).

Drinkers	LVD		HVD		*P*-values		
feeders	LVF	HVF	LVF	HVF	D	F	D x F
Inactive behaviours							
Lying inactive (eyes open)	0.321^{b} ± 0.012	0.320^{b} ± 0.012	0.388^{a} ± 0.013	0.372^{ab} ± 0.012	**0.0027**	0.40	**0.0021**
Lying inactive (eyes closed)	0.141 ± 0.009	0.119 ± 0.012	0.129 ± 0.009	0.104 ± 0.012	0.41	**0.024**	0.54
Standing inactive	0.061 ± 0.004	0.069 ± 0.005	0.043 ± 0.003	0.043 ± 0.003	0.051	0.77	0.18
Social behaviours							
Nosing pen mates	0.119^{ab} ± 0.005	0.141^{a} ± 0.007	0.099^{b} ± 0.004	0.109^{ab} ± 0.005	**0.043**	**0.0026**	**0.024**
Aggressing pen mates	0.007^{b} ± 0.001	0.014^{a} ± 0.002	0.010^{ab} ± 0.001	0.014^{ab} ± 0.002	0.83	**0.0081**	**0.036**
Manipulating pen mates	0.018 ± 0.002	0.017 ± 0.003	0.023 ± 0.002	0.024 ± 0.003	**0.0031**	0.50	0.23
Exploratory behaviours							
Manipulating enrichments	0.029 ± 0.003	0.029 ± 0.004	0.027 ± 0.003	0.034 ± 0.004	0.60	0.91	0.053
Exploring the pen	0.129^{a} ± 0.006	0.121^{ab} ± 0.007	0.112^{b} ± 0.005	0.133^{ab} ± 0.006	0.92	0.51	**0.036**
Locomotion behaviours							
Walking	0.005 ± 0.001	0.006 ± 0.001	0.004 ± 0.001	0.006 ± 0.001	0.82	0.54	0.27
Maintenance behaviours							
Eating	0.111 ± 0.006	0.103 ± 0.008	0.112 ± 0.006	0.114 ± 0.007	0.72	0.30	0.83
Drinking	0.031 ± 0.002	0.029 ± 0.003	0.029 ± 0.002	0.024 ± 0.002	0.43	0.40	0.20
Eliminating	0.007 ± 0.001	0.005 ± 0.001	0.010 ± 0.002	0.009 ± 0.002	0.26	0.78	0.58

Data are presented as proportions of total scan observations (means ± SEM). The significant main effects of contact voltage in drinkers, contact voltage in feeders or their interactions are indicated in **bold** ($P \leq 0.05$). Different letters denote differences for interactions (D x F) and indicate significant difference at $P < 0.05$. D: drinkers. F: feeders. HV: high voltage. LV: low voltage.

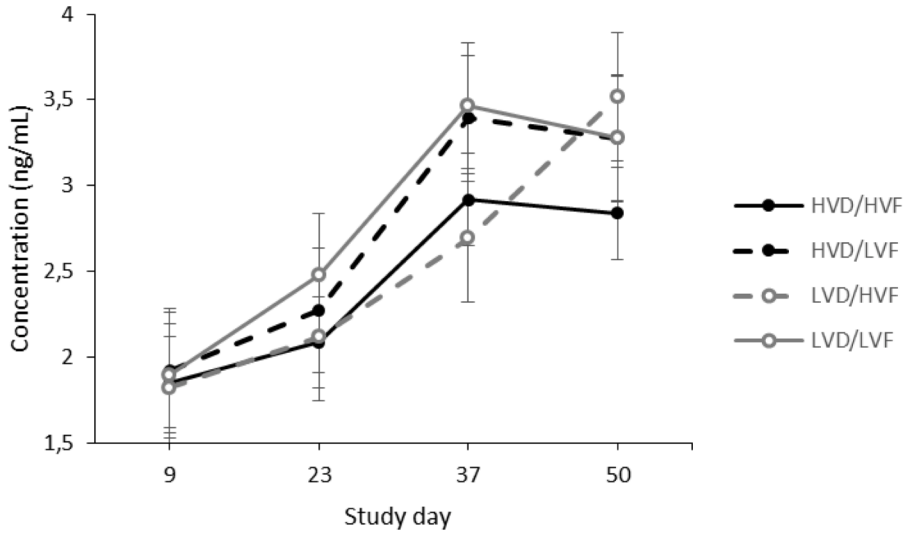

Fig. 2. Mean (± SD) pooled salivary cortisol concentration by study day. HVD = high voltage in drinkers (> 125 mV); LVD= low voltage in drinkers (≤ 125 mV); HVF= high voltage in feeder (> 50 mV); LVF= low voltage in feeder (≤ 50 mV).

Table 3
Salivary and hair cortisol, blood concentrations of haptoglobin, hydroperoxides (HPO), blood antioxidant capacity (BAP) and oxidative stress index (OSI) during the trial.

Drinkers	**LVD**		**HVD**		***P*-values**		
feeders	**LVF**	**HVF**	**LVF**	**HVF**	**D**	**F**	**DxF**
Cortisol							
Salivary cortisol[a] (ng/mL)	3.0 ± 1.1	2.6 ± 0.8	2.9 ± 1.2	2.6 ± 0.7	0.31	0.075	0.071
Hair cortisol (ng/g)	12.7 ± 2.1	12.8 ± 2.6	12.4 ± 2.8	13.6 ± 3.1	0.77	0.090	0.18
Blood parameters							
Haptoglobin (g/L)	1.8 ± 1.5	1.9 ± 1.5	1.9 ± 1.5	2.2 ± 1.7	0.29	0.16	0.38
HPO (CARRU)	1214.8 ± 225.1	1205.1 ± 218.3	1215.7 ± 238.9	1333.2 ± 250.9	**0.039**	0.06	0.06
BAP (µmol/L eq Vit C)	2726.1 ± 266.6	2770.2 ± 264.2	2775.7 ± 273.1	2781.1 ± 289.8	0.10	0.73	0.45
OSI (CARRU.µmol^{-1}.L eq Vit C)	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.14	0.092	0.086

Data are presented as the mean ± SDs for each parameter, all time points pooled. Significant main effects of contact voltage in drinkers, contact voltage in feeders or their interactions are indicated in **bold** ($P \leq 0.05$). D: drinkers. F: feeders. HVD= high voltage in drinkers (> 125 mV); LVD= low voltage in drinkers (≤ 125 mV); HVF= high voltage in feeder (> 50 mV); LVF= low voltage in feeder (≤ 50 mV).

[a] For salivary cortisol, the pen was the experimental unit. For the other parameters, the pig was the experimental unit.

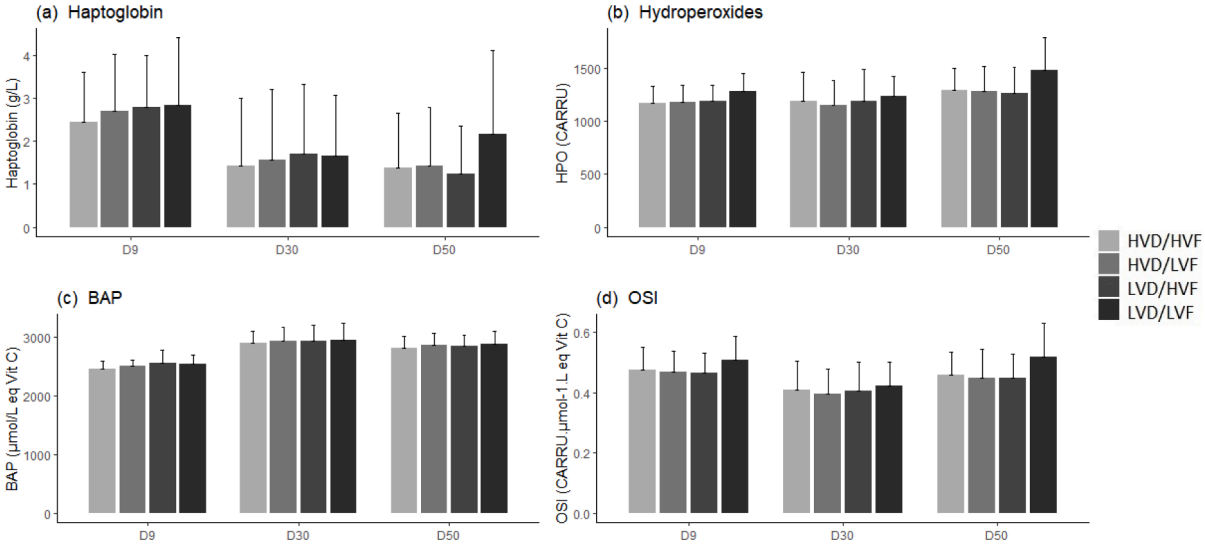

Fig. 3. Plasma concentration of haptoglobin (a), hydroperoxides (HPO) (b), blood antioxidant capacity (BAP) (c) and mean oxidative stress index (OSI) (d) on days 9, 30 and 50 of the study. All data are reported as the mean (±SD) of results obtained for each group. HVD= high voltage in drinkers (> 125 mV); LVD= low voltage in drinkers (≤ 125 mV); HVF= high voltage in feeder (> 50 mV); LVF= low voltage in feeder (≤ 50 mV).

potential. Sources include (without being exhaustive) lights, variable frequency drives in motors (such as fans and grain grinders for example), water pumps, and radio frequency monitoring systems. Therefore, continuous recording of voltage and frequency should be implemented in further research. These daily variations in stray currents could also potentially explain some of the low effects we observed during our experiment on pigs' behaviours and biomarkers, which we discussed below.

Regarding the effects of stray currents in the feeders, we observed that piglets exposed to stray currents of over 50 mV in the feeders spent less time resting (lying with their eyes closed), which may suggest that in this context piglets are less relaxed and more stressed. It may reflect a greater need for vigilance against pen mates (Beattie et al., 2000). They also spent more time aggressing their pen mates. This effect was only visible when the voltage in the drinker was low, probably because, high voltages in the drinker also tended to disturb animal behaviour and blurred the observations on aggressive behaviour. Aggressive behaviours is expressed immediately after mixing when the group hierarchy must be established (Bolhuis et al., 2005; McGlone, 1986), but if it continues over time, aggression between piglets suggests situations of discomfort, irritability, annoyance or frustration (Giersing and Studnitz, 1996; Langbein and Puppe, 2004; Ruis et al., 2002). As our behavioural observations started 8 days after weaning, we can assume that the hierarchy was by then established and that the presence of stray currents in the environment induced such a situation. However, these results should be interpreted with caution since aggressive behaviours (fighting, head-butting and biting with avoidance of the pen mate) could have been in some cases mistaken from play behaviours, particularly using scan observations.

Regarding the effects of currents in the drinkers, we observed less nosing in the presence of stray currents of over 125 mV in the drinkers, although this effect was somewhat blurred by the voltage level in the feeders. Nosing pen mates is considered socio-positive as it appears to be involved in group cohesion and social recognition mechanisms (Camerlink and Turner, 2013). Thus, less nosing could be detrimental to the social interactions among pen mates. Piglets exposed to stray currents of over 125 mV in drinkers also spent more time manipulating pen mates. In piglets, oral manipulations of the body of the pen-mates, most often of the ear or tail, are indicators of poor welfare. They are for example observed in response to the distress due to early artificial rearing (Schmitt et al., 2019) or frustration due to impossibilities to perform oral manipulation directed to an adequate substrate like a teat for younger pigs or a foraging substrate in older ones (Warns et al., 2021). Uneasiness associated to an inflammatory state can also favour ear manipulation by the sick piglet (Veit et al., 2021). For piglets that were not additionally exposed to currents in the feeder, the presence of stray currents in the drinkers also decreased the time spent exploring the environment. Environmental exploration is an important behaviour for pigs as they spend around 70% of their active time expressing this behaviour under optimal welfare conditions (Stolba and Wood-Gush, 1989; Studnitz et al., 2007). Overall, in the presence of stray currents, we observed a decrease in resting time, an increase of time spent inactive in alert (lying with eyes open), an increase in socio-negative behaviours (aggression and oral manipulation) and a decrease in socio-positive behaviours (nosing). Thus, although further studies are needed to confirm our hypotheses, these negative behavioural effects suggest that pigs can sense stray currents of low voltage in the housing environment, and that these currents have a moderate detrimental effect on their welfare.

Besides behavioural indicators, in our study, we decided to measure cortisol concentration in saliva at the pen level and in bristles at the individual level (Merlot et al., 2011; Thomsson et al., 2014). These two methods have the advantage of being non-invasive. Salivary cortisol reflects the immediate secretion of cortisol, and is then related to the emotional state of the animal at the time of sampling (Merlot et al, 2011). To limit the influence of circadian rhythm, all samples were collected at the same hour at each sampling day. Cortisol accumulation in pig bristles reflects cumulative HPA axis activity during the hair growth period (Casal et al., 2017; Heimbürge et al., 2019, 2020a; Prims et al., 2019). So, cortisol levels in bristles reflected the preceding period with increased cortisol release and may be a promising indicator of chronic stress (Heimbürge et al., 2020b). One limitation to this measure is that body region and hair color affect hair cortisol concentration (Heimbürge et al., 2020a) and collecting a sufficient quantity of hair meant shaving a large area on the piglets' backs. Hair and salivary cortisol concentration indicated that stray currents did not influence cortisol secretions, which is in agreement with previous reports in cattle (Rigalma et al., 2010). Since variations in cortisol secretion are induced by high-intensity emotions (Moscovice et al., 2022), this result is also in agreement with the moderate effects that we observed on the behaviour of the pigs.

The increase in HPO observed along time during our study was previously reported and might reflect a high rate of reactive oxygen species production resulting from a high cellular activity for tissue accretion (Buchet et al., 2017). The possible pro-oxidant effect of growth is supported by the observation that fast growing lines of pigs, like those studied in this trial, have deteriorated redox status compared to slow growing pigs (Brambilla et al., 2002; Merlot et al., 2012). Since psychological stress can also generate oxidative stress (Durand et al, 2022), this increase in HPO could also be linked to an increase in the stress due to the growing discomfort associated with piglets' growth, and thus the increase in animal density. However, we observed that the presence of stray currents in drinkers also increased blood HPO concentrations. Furthermore, the effects of stray voltages in the feeders on HPO was not far from statistical significance, suggesting additional effect of high voltages in the feeder and the drinkers. This is supported by previous reports in other species where higher currents can generate an oxidative stress (Consales et al., 2012). This effect is based on the alteration by electromagnetic mechanisms of chemical reactions in the cells, among which those regulating metabolic and redox reactions (Consales et al, 2012).

In our study, the concentration in haptoglobin decreased over time during the experiment. The literature reports a trend towards an increase in haptoglobin with age in growing pigs (Piñeiro et al., 2009), particularly from the time of transfer to fattening unit, i.e. a period after the end of our study. Here, the greater levels at the beginning of the trial could be explained because weaning induces a systemic inflammation, which may be measured using haptoglobin as previously reported (Sauerwein et al., 2005). We did not observe any difference between groups in blood haptoglobin concentration. So, although the stray currents generated oxidative stress, they did not have a sufficient effect to generate inflammation. These physiological results also confirm that the stress caused by stray currents of under 500 mV during our experiment remained at a moderate level.

Regarding practical advices when dealing with suspicious stray currents in piggeries, on-farm sources of stray voltages are frequently multiple. The first step is to monitor and determine the sources of stray voltages. Then, stray voltages generated by on-farm equipment may be remediated by checking the proper grounding of the electrical system, or by disconnecting or replacing the offending equipment, or by filtering the high-frequency transients generated by the equipment. Off-farm sources are more challenging to remediate since the electricity provider and sometimes local authorities must be associated in the process.

5. Conclusion

Stray currents of low voltage in drinkers and feeders in the post-weaning unit affected the behaviour of weaned piglets. We observed a decrease in resting time, an increase of time spent inactive in alert, an increase in socio-negative behaviours and a decrease in socio-positive behaviours in the presence of stray currents in housing, which may reflect altered welfare for piglets. Moreover, the blood oxidative status

of piglets was also increased, particularly in pigs exposed via drinkers. This observational field study showed that stray currents of less than 0.5 V in the pig's environment generate moderate behavioural and physiological stress responses, and suggest that they might affect the health and welfare of pigs. This study is a proof-of-concept and the first step for other trials with the purpose of gaining knowledge about the complex and controversial topic of stray currents.

CRediT authorship contribution statement

Théo Nicolazo: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Writing – original draft, Writing – review & editing. **Elodie Merlot:** Writing – original draft, Conceptualization, Methodology, Writing – review & editing. **Charlotte Teixeira Costa:** Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **Caroline Clouard:** Conceptualization, Writing – review & editing. **Arnaud Lebret:** Investigation, Writing – review & editing. **Céline Chevance:** Investigation, Conceptualization, Methodology, Writing – review & editing. **Valérie Normand:** Investigation, Writing – review & editing. **Justine Jeusselin:** Investigation, Writing – review & editing. **Gwenaël Boulbria:** Investigation, Methodology, Project administration, Writing – original draft, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.livsci.2024.105555.

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