Nursery pig behavior evaluation pre- and post injection using digital-image methodology

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Summary

Objectives: To determine if nursery pigs display different behaviors and postures pre- and post injection during the human-approach paradigm using a digital photographic image.

Materials and methods: A digital camera captured an image of nursery pigs in a pen during a human-approach paradigm at two time points, pre- and post injection, with three different treatments. A total of 149 pens containing crossbred, mixed-sexed nursery pigs 42 days of age were used. Each pen of pigs was randomly assigned to one of three injection treatments: Vaccine A (saline administered on day 28 and Vaccine A on day 43); Vaccine B (vaccine administered days 28 and 43); and saline (VSAL; saline administered on days 28 and 43). All pigs were classified as Touched, Oriented, or Not Oriented. Pigs classified as Not-Oriented were further delineated into four postures and two behaviors. Within behavioral categories, snout and tail-base distances from the human were measured.

Results: There were no pre-injection pen behavioral differences. Fewer Vaccine B-treated pens were classified as Touched compared to Vaccine A- and VSAL-treated pens. Regardless of treatment, more pigs were Not Oriented post injection than pre-injection. Fewer Vaccine B-treated pigs stood than did other treatments. Vaccine B-treated pigs had the greatest snout and tail-base distances from the human.

Implication: It is important to establish the age of the nursery pigs and the vaccine with which they are treated when conducting an on-farm assessment using a human-approach paradigm.

Keywords: swine, human-approach paradigm, injection, behavior

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Resumen - Evaluación de la conducta de cerdos en destete pre y post inyección utilizando una metodología de imagen digital

Objetivos: Determinar si los cerdos en destete exhiben diferentes conductas y posturas pre y post inyección durante el paradigma de acercamiento humano utilizando una imagen fotográfica digital.

Materiales y métodos: Una cámara digital capturó una imagen de cerdos de destete en corral durante el paradigma de acercamiento humano en dos momentos: pre y post inyección, con tres tratamientos diferentes. Se utilizaron un total de 149 corrales que contenían cerdos de destete, híbridos, de ambos sexos, de 42 días de edad. Cada corral de cerdos se asignó aleatoriamente a uno de tres tratamientos de inyección: Vacuna A (solución salina administrada en el día 28 y Vacuna A en el día 43); Vacuna B (vacuna administrada en los días 28 y 43); y solución salina (VSAL [por sus siglas en inglés]; solución salina en los días 28 y 43). Todos los cerdos fueron clasificados como Tocados, Orientados, o No Orientados. Los cerdos clasificados como No Orientados se definieron en cuatro posturas y dos conductas. Dentro de las categorías conductuales, se midió la distancia entre el hocico y la base de la cola, y el humano.

Resultados: No hubo diferencias conductuales de corral pre inyección. Se clasificaron menos corrales tratados con la Vacuna B como Tocados comparado contra los corrales tratados con la Vacuna A y VSAL. Independientemente del tratamiento, hubo más cerdos No Orientados post inyección que pre inyección. Menos cerdos tratados con la Vacuna B permanecieron quietos que en los otros tratamientos. Los cerdos tratados con la Vacuna B presentaron la mayor distancia entre el hocico y la base de la cola y el humano.

Implicacione: Es importante establecer la edad de los cerdos en destete y la vacuna con la que son tratados cuando se realiza una valoración en granja utilizando un paradigma de acercamiento humano.

Résumé - Évaluation des porcelets en pouponnière pré- et post-injection à l'aide d'une méthodologie par image digitale

Objectifs: Déterminer si des porcelets en pouponnière démontrent des comportements différents ainsi que leur posture pré- et post-injection durant le paradigme d'une approche humaine à l'aide d'images photographiques digitales.

Matériels et méthodes: Une caméra digitale enregistra une image de porcelets en pouponnière dans un enclos durant un paradigme d'une approche humaine à deux

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moments dans le temps: pré- et post-injection, avec trois traitements différents. Au total, 149 enclos hébergeant des porcelets en pouponnière de race croisée, appartenant aux deux sexes et âgés de 42 jours ont été utilisés. Chaque enclos de porcelets a été assigné au hasard à l'un des trois traitements par injection: Vaccin A (saline administrée au jour 28 et Vaccin A au jour 43); Vaccin B (vaccin administré aux jours 28 et 43); et saline (VSAL; saline administrée aux jours 28 et 43). Tous les porcs ont été classés en tant que Touché, Orienté, ou Non-Orienté. Les animaux classés comme Non-Orienté ont subséquemment été définis selon quatre postures et deux comportements. À l'intérieur des catégories de comportement, les distances du groin et de la base de la queue par rapport à un humain ont été mesurées.

Résultats: Il n'y avait pas de différence dans le comportement pré-injection parmi les enclos. Moins d'enclos parmi le groupe Vaccin B furent classés comme Touché comparativement à ceux des groupes Vaccin A et VSAL. Indépendamment du traitement, plus de porcelets étaient Non-Orienté post-injection que pré-injection. Moins de porcelets du groupe Vaccin B se tenaient debout comparativement aux autres traitements. Les porcelets du groupe Vaccin B avaient les plus grandes distances du groin et de la base de la queue par rapport à l'humain.

Implication: Il est important d'établir l'âge des porcelets en pouponnière et le vaccin avec lequel ils seront traités lorsque l'on mène une évaluation sur la ferme en utilisant le paradigme d'une approche humaine.

On-farm welfare assessments and third-party audits are carried out to document compliance with animal care and welfare policies and procedures.[1] Welfare assessment and audit criteria can be divided into resource- and animal-based measures. One animal-based measure is the human-approach paradigm (HAP). The aim of this paradigm is to determine the animal-human relationship, ie, positive, neutral, or negative. The Welfare Quality Assurance program assesses this paradigm;[2] however, the Pork Quality Assurance Plus (PQA-Plus) Program and the Common Swine Industry Audit (CSIA) describe the importance of pig-human interactions, but do not formally assess or audit the paradigm.[3] The predecessor to PQA-Plus, SWAP (Swine Well-being Assurance Program), did include a HAP. When assessed for validity, the HAP was amended to be a bench-marking evaluation instead of a required assessment due to inconsistent repeatability attributed to differing production strategies. Preliminary work using the HAP noted that nursery pigs recently vaccinated with porcine circovirus type 2 (PCV2) were reluctant to approach a human in their home pen.[4] Vaccines are extremely important to protect pig health and improve welfare, but pigs not approaching the human because they were recently injected (vaccinated) could be misinterpreted as being poorly handled. The method of collecting information during the HAP is also an important consideration. Previous work by Weimer et al[1] compared live observation to a digital photographic image. The major benefit of a digital photographic image is the infinite amount of time available for retrospective analysis. Hence, if we could determine the nursery pig's behavioral changes pre- and post injection during the HAP using a digital photographic image, this may better define the effect of vaccination to support conclusions based on behavior. Therefore, the objective of this study was to determine if nursery pigs display different behaviors and postures pre- and post injection during the HAP recorded using digital photographic images.

Materials and methods

All procedures were approved by the Iowa State University Animal Care and Use Committee.

Animal care and husbandry protocols for this experiment were overseen by the company veterinarian and farm manager. These protocols were based on the US swine industry guidelines presented in the Pork Quality Assurance Plus.[3]

Animals

The experiment was conducted in November 2011 at a commercial nursery site located in South Central Missouri. Crossbred PIC barrows and gilts (housed in mixed pens) were 42 days of age and weighed approximately 12 kg when the experiment began. Pigs were not individually weighed before the experiment.

Housing and management

A total of 149 pens (averaging 20 pigs per pen, 2991 pigs total) distributed in four rooms were used in this study. Rooms measured 34.1 m width × 18.3 m length, and ceiling height was 2.1 m. Pens measured 1.8 m width × 3 m length, providing 0.3 m^2 per pig, and all pens had woven wire flooring (3-gauge Boss Hog; J & L Wire, St Paul, Minnesota). A stainless steel rectangular feeder (Automated Production Systems, Assumption, Illinois) was located either on the right or left side of the pen. Pigs were provided ad libitum access to a pelleted diet (1549 kcal per kg metabolizable energy and 22% crude protein) formulated to meet or exceed National Research Council nutrient requirements by each nursery phase.[5] Each pen contained one stainless steel nipple drinker (Drik-O-Mat; Egebjerg, Denmark). Fifteen incandescent lights were turned on at 8:00 AM for daily chores and then were turned off at 11:00 PM. Rooms were mechanically ventilated with a curtain system, two stir fans, 10 inlets, and two heaters (Re-Verber-Ray; Detroit Radiant Products Company, Warren, Michigan). Daily temperatures were recorded using data loggers (HOBO H08-003-02; Onset Data Loggers, Bourne, Massachusetts). Caretakers observed all pigs twice daily.

Injection treatment

The pen-applied injection treatments were Ingelvac CircoFLEX-Ingelvac MycoFLEX vaccine (Vaccine A; Boehringer Ingelheim Vetmedica Inc, St Joseph, Missouri); Circumvent PCVM vaccine (Vaccine B; Merck, Kenilworth, New Jersey); and Saline (VSAL; Hyclone Phosphate Buffered Saline; Sigma Aldrich, St Louis, Missouri).

Experimental design

The experimental unit was the pen of pigs and an entire pen of pigs received the same injection treatment. Injection treatments applied to each pen were completely randomized and blocked within four rooms so that injection order did not affect the behavioral outcomes. On arrival at the nursery (28 days of age), pigs given Vaccine B received their first Vaccine B dose. Pigs assigned the Vaccine A treatment received saline. Pigs assigned the VSAL treatment received Vaccine A (the farm's health program required pigs to have vaccination coverage; Table 1). When pigs were 42 days of age, pig behavior was collected at 4:00 PM (pre-injection). At 43 days of age, pigs were given their assigned second injection treatment beginning at 10:00 AM, and then behavior was collected beginning at 4:00 PM (post injection).

Table 1: Injection treatments given to nursery pigs in a study of behavioral changes pre- and post injection*

Age (days)	Injection treatment† Vaccine A	Vaccine B	VSAL
28	VSAL	Vaccine B	Vaccine A
43	Vaccine A	Vaccine B	VSAL

* Commercial pens measuring 1.8 m width × 3 m length provided 0.3 m^2 space per pig. PIC barrows and gilts (housed in mixed pens) were administered the first injection treatment at 28 days of age. When pigs were 42 days of age, behavior was collected at 4:00 PM (pre-injection). At 43 days of age, pigs were given their second assigned injection treatment beginning at 10:00 AM, and then behavior was collected beginning at 4:00 PM (post injection).

† Pens of pigs were treated either with Vaccine A (CircoFLEX/MycoFLEX 2-mL dose; Boehringer Ingelheim Vetmedica, Inc, St Joseph, Missouri; n = 48 pens), or Vaccine B (Circumvent-PCVM 2-mL dose; Merck, Whitehouse Station, New Jersey; n = 51 pens), or phosphate buffered saline (Hyclone Phosphate Buffered Saline 2-mL dose; Sigma Aldrich, St Louis, Missouri; VSAL; n = 50 pens), each administered as a single intramuscular dose injected into the right lateral cervical musculature using a 16-gauge needle.

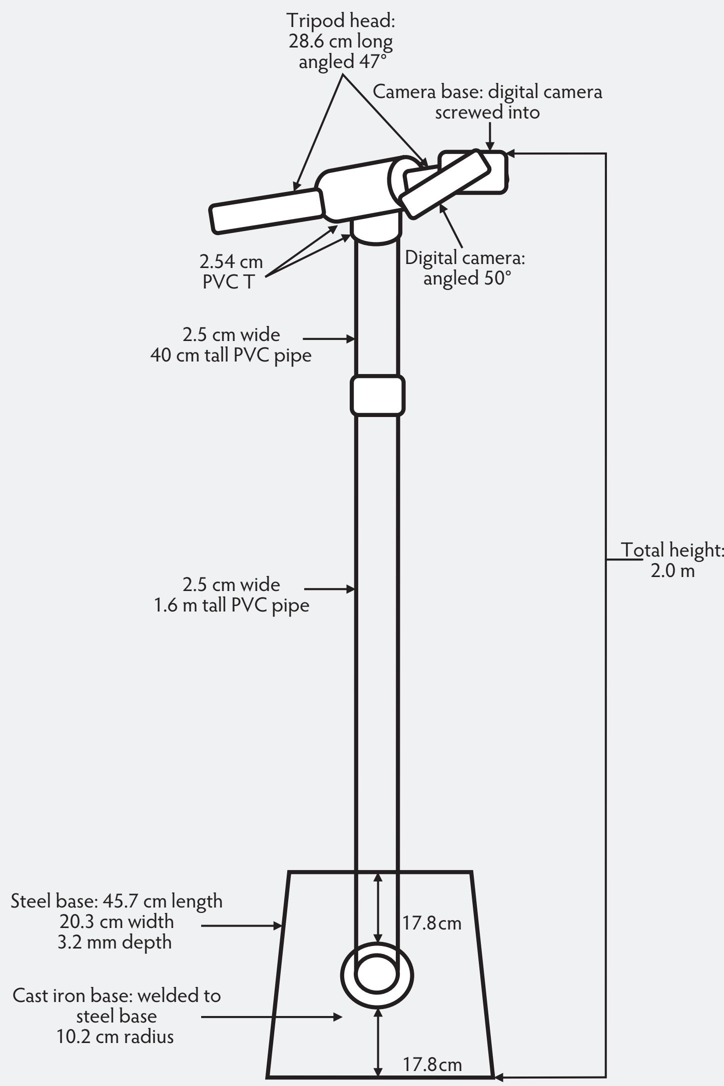
Injection methodology

Pigs were moved towards the alley end of their home pen by the farm manager using a sort board. Pigs were not picked up and individually handled in an effort to avoid any additional handling stressors.[6,7] The site owner-manager and the pig owner administered the preset dose using a Uni-Matic 2-mL, multi-dose syringe (approximately 1 second per pig) into the lateral cervical musculature on the right side of the neck using a 16-gauge, 1.6-cm length needle (Air-Tite Products Co Inc, Virginia Beach, Virginia). To avoid injecting the same pig twice, a mark was placed between the pig's scapulas using an animal-safe crayon after injection (Raidex Animal Marking Sticks; Thousand Hill Supply, Walworth, New York). The same personnel performed injection treatments for all pigs. Injection treatments were administered to pigs within pens in an alternating fashion across the alleyway.

Digital photograph system

The digital photograph system was constructed using similar methods to those previously described by Weimer et al.[1] Briefly, the digital photograph system was free standing and positioned in the alleyway at the midpoint of the adjacent front pen gate where there were no feeder obstructions, and the image captured the entire nursery pen (Figure 1). The camera (Pentax Optio W90 model; Pentax Imaging Company, Golden, Colorado) was equipped with an infrared wireless shutter remote control (Pentax Imaging Company) to record the images while the observer was in the nursery pen. The camera focal length was 28 mm, with a 3-megapixel resolution.

Figure 1: Digital photograph system schematic used to capture the pig images within each pen (1.8 m width × 3 m length).



Human-approach paradigm

The HAP (Figure 2) was applied to pens in the order that injection treatments were administered. Upon entry into the room, the observer and digital photograph system operator walked down the length of the nursery room to the farthest pen. The observer positioned the digital photograph system at the midpoint of the adjacent pen front gate. The observer stepped over the gate and entered the nursery pen, immediately crouching with head down at gate center. Simultaneously, the digital photograph system operator sat on a bucket in the alleyway, directly behind the crouched observer, and leaned back on the gate. The observer extended and held still the left leather-gloved hand with the index finger extended, and began a stopwatch, avoiding eye contact with pigs for 15 seconds. The left hand and finger were extended to allow the same anatomical location to be clearly visible on each digital image. At the end of the 15 seconds, the observer signaled to the digital photograph system operator by leaning back against the gate, and the system operator captured an image of the pen using a wireless remote. The HAP methodology was completed quietly, with no talking between the observer and the digital photograph system operator.

Behavior classification

The same observer that conducted the HAP on-farm in each pen also analyzed each digital image taken by the digital image photography system operator. The observer was blinded to vaccine treatments until all images had been analyzed. Within each digital image of individual pens, all pigs were classified into three categories, Touched, Oriented, or Not Oriented, at the ISU-Animal Behavior Laboratory using Adobe Photoshop CS5 (Adobe Systems Inc, San Jose, California; Figure 2). Not Oriented pigs were further classified into four mutually exclusive postures or two behaviors (Table 2).

For both pre- and post injection treatments, pig percentages for Touched, Oriented, and Not Oriented categories were calculated as [No. of pigs categorized as Touched or Oriented or Not Oriented in the pen ÷ Total no. of pigs in the pen] × 100.

For both pre- and post injection treatments, pig percentages for further delineating Not Oriented postures or behaviors (standing, sitting, piling, lying, head in feeder, and

Figure 2: Examples of nursery pigs classified as Touched (numbers 1, 2, 3, 4, and 5), Oriented (numbers 8, 9, and 10), and Not Oriented (numbers 6, 7, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, and 21). The observer positioned the digital photograph system at the midpoint of each pen gate in the alleyway. The HAP was performed on each pen of pigs by the observer stepping into the pen and immediately crouching with head down at the center of the gate. The digital photograph system operator sat on a bucket on the opposite side of the gate. The observer extended and held still the left hand for 15 seconds. After 15 seconds, the observer signaled to the digital photograph system operator by leaning against the front gate, at which point the digital image of the pen was captured.

mouth around drinker) were calculated as [No. of pigs categorized in further delineated Not Oriented postures or behaviors in the pen ÷ Total no. of pigs categorized as Not Oriented in the pen] × 100.

The percentage difference was calculated by subtracting the post injection percentage of pigs from the pre-injection percentage of pigs categorized as Touched, Oriented, Not Oriented, and Not Oriented further delineated postures or behaviors.

Snout and tail-base distance. Distance (cm) from the human observer's left index finger in the pen to the snout and tail-base of each pig was measured using the digital image (Figure 2). Snout and tail-base anatomical locations were chosen from previous work conducted by Weimer et al.[1] Snout was defined as the midpoint of the superior snout, and tail-base was defined as the point of the pig's superior rear located at the tail-base. If a pig snout or tail-base was not visible in the digital image, the distance was excluded from the final data set. Snout and tail-base proximities were measured using the ruler tool in Adobe Photoshop CS5 (Adobe Systems Inc). To determine the actual snout distance, lengths collected from the digital image using the Adobe ruler were converted

Table 2: Nursery pig behavior classification using a digital image analysis at the conclusion of a human approach paradigm (HAP)*

Measure	Definition
Touched	Any part of the pig's body touching the human observer.
Oriented	Pig Oriented toward the human. Using Adobe Photoshop (Adobe Systems Incorporated, Arden Hills, Minnesota), in the digital image, a line was drawn from the midpoint between the pig's eyes to the center of the snout and extended towards the edge of the pen. If the line intersected with the human, the pig was classified as Oriented.
Not Oriented	Pigs not exhibiting the above two behavioral classifications.
Standing	Upright position with all four feet on the floor
Sitting	Hind legs and buttocks touching the floor
Piling	Two or more feet off the floor with body atop a pen mate
Lying	Sternal or lateral body contact with the floor
Head in feeder	Pig's head is inside the feed trough
Mouth around drinker	Pig's mouth encircles the nipple drinker

* Ethogram based on Weimer et al.[1]

using the actual length of the nursery feeder (90.4 cm) and the feeder radius in pixels (620 pixels) for the digital image. The nursery feeder in the image was chosen as the calibration focus for the ruler tool because it was always visible and consistently the same length in each pen. The conversion ratio was 6.9 (620 pixels ÷ 90.4 cm = 6.9). It was possible to collect 2863 of 5982 total snout and tail-base distance measures.

Statistical analysis. All data were analyzed using SAS software version 9.3 (SAS Institute Inc; 2011). The three behavioral categories (Touched, Oriented, and Not Oriented), the Not Oriented postures and behaviors, and the snout and tail-base distance to the observer's index finger were analyzed for normal distribution before analysis with PROC UNIVARIATE. The treatment comparisons pre- and post injection, as well as the differences (calculated by subtracting the post injection percentage of pigs from the pre-injection percentage), within behavioral categories were not normally distributed and hence data were analyzed using a generalized mixed linear model (PROC GLIMMIX). The snout and tail-base distances to the observer's index finger were normally distributed and were analyzed using a mixed linear model (PROC MIXED). For both models, the fixed effect of injection treatment (Vaccine A, Vaccine B, and VSAL), with the random effects of pen nested within room were used. A value of $P < .05$ was considered significant and differences between means were detected using PDIFF.

Results

Behavior

There were no pre-injection treatment differences for Touched, Oriented, and Not Oriented ($P \geq .22$). Post injection, fewer Vaccine B-injected pens of pigs were classified as Touched compared to Vaccine A and VSAL-injected pens ($P < .001$). More pens of saline-injected pigs were classified as Oriented compared to pens of Vaccine A- and Vaccine B-injected pigs ($P < .001$; Table 3). When comparing pen pre- and post injection differences, fewer Vaccine B pigs were classified as Touched (-6.9%; $P < .05$; Figure 3). All pen-applied injection treatments had fewer pigs classified as Oriented (range -0.3% to -10%; $P < .05$; Figure 3) post injection. There was a 17% average pen increase for Vaccine B-injected pigs classified as Not Oriented ($P < .05$; Figure 3). There were no pre-injection treatment differences observed for the percentages of pigs within each pen classified as standing, sitting, piling, lying, head in feeder, and mouth around drinker ($P > .05$; Figure 4). Post injection, there were fewer Vaccine B-injected pens of pigs classified as standing ($P < .001$), but more were classified as sitting ($P < .001$) and lying ($P < .01$) compared to pens receiving Vaccine A and VSAL injection treatments (Table 4). Regardless of injection treatment, the pre- and post injection differences resulted in more pigs classified as lying and fewer standing within each pen ($P < .05$; Figure 4).

Snout and tail-base distance

There were no pre-injection differences observed for snout and tail-base distances between pen treatment groups for pigs classified as Touched, Oriented, or Not Oriented ($P \geq .13$). Post injection, there were no injection treatment differences for the snout or tail-base average distances in the Touched and Not Oriented categories ($P \geq .10$). However, the average distances between the pigs' snout and tail-base in relation to the observer's left index finger were shorter for pens that received the Vaccine A injection treatment ($P < .05$) than for pens that received the Vaccine B treatment in the Oriented category (Table 5).

Discussion

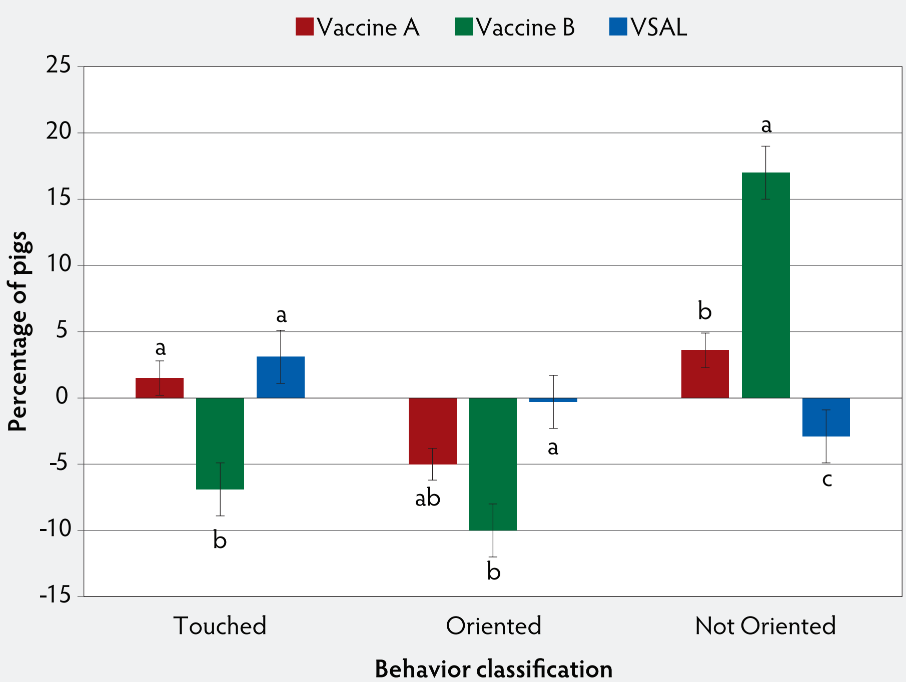
In 2004, porcine circovirus disease (PCVD) emerged.[8] When commercial pigs were exposed to this viral pathogen, mortalities were reported at over 20%. By 2006, two vaccination products were available: Suvaxyn, (Fort Dodge Animal Health, Fort Dodge, Iowa) a chimera product, and Circumvent PCV2, (Merck, Kenilworth, New Jersey), a subunit vaccine. Swine producers observed a transient behavioral change among pigs treated with the PCV2 vaccines,

Table 3: Behavioral pen mean percentages (± SE) of commercial nursery pigs in a human approach paradigm (HAP) classified as Touched, Oriented, or Not Oriented pre- and post injection*

	Injection treatment			
	Vaccine A	Vaccine B	VSAL	
No. of pens	**48**	**51**	**50**	***P***
Pre-injection				
Touched†	9.8 ± 1.0	12.0 ± 1.6	10.0 ± 1.2	.54
Oriented	32.8 ± 1.8	34.5 ± 1.8	33.5 ± 1.8	.80
Not Oriented	57.3 ± 1.6	53.5 ± 1.6	56.6 ± 1.6	.22
Post injection				
Touched	11.3 ± 1.2[a]	5.1 ± 1.5[b]	13.1 ± 1.5[a]	< .001
Oriented	27.8 ± 1.5[b]	24.4 ± 1.5[b]	33.2 ± 1.5[a]	< .001
Not Oriented	60.9 ± 1.7[b]	70.5 ± 1.7[a]	53.7 ± 1.7[c]	< .001

* Vaccines and schedules described in Table 1.
† Ethogram of behaviors described in Table 2.
[abc] Means within a row with no common superscript are significantly different (LS Means; *P* < .05).

Figure 3: Behavioral pen mean percentage (± SE) differences (calculated by subtracting the post-injection percentage of pigs from the pre-injection percentage of pigs) for commercial nursery pigs classified as Touched, Oriented, and Not Oriented (behaviors described in Table 2) using a digital image at the conclusion of the human approach paradigm (HAP; methodology described in Figure 2) pre- and post injection (vaccines and schedules described in Table 1). Means were compared within a behavior category. Means with no common superscript [(abc)] are significantly different (LS Means, *P* < .05).

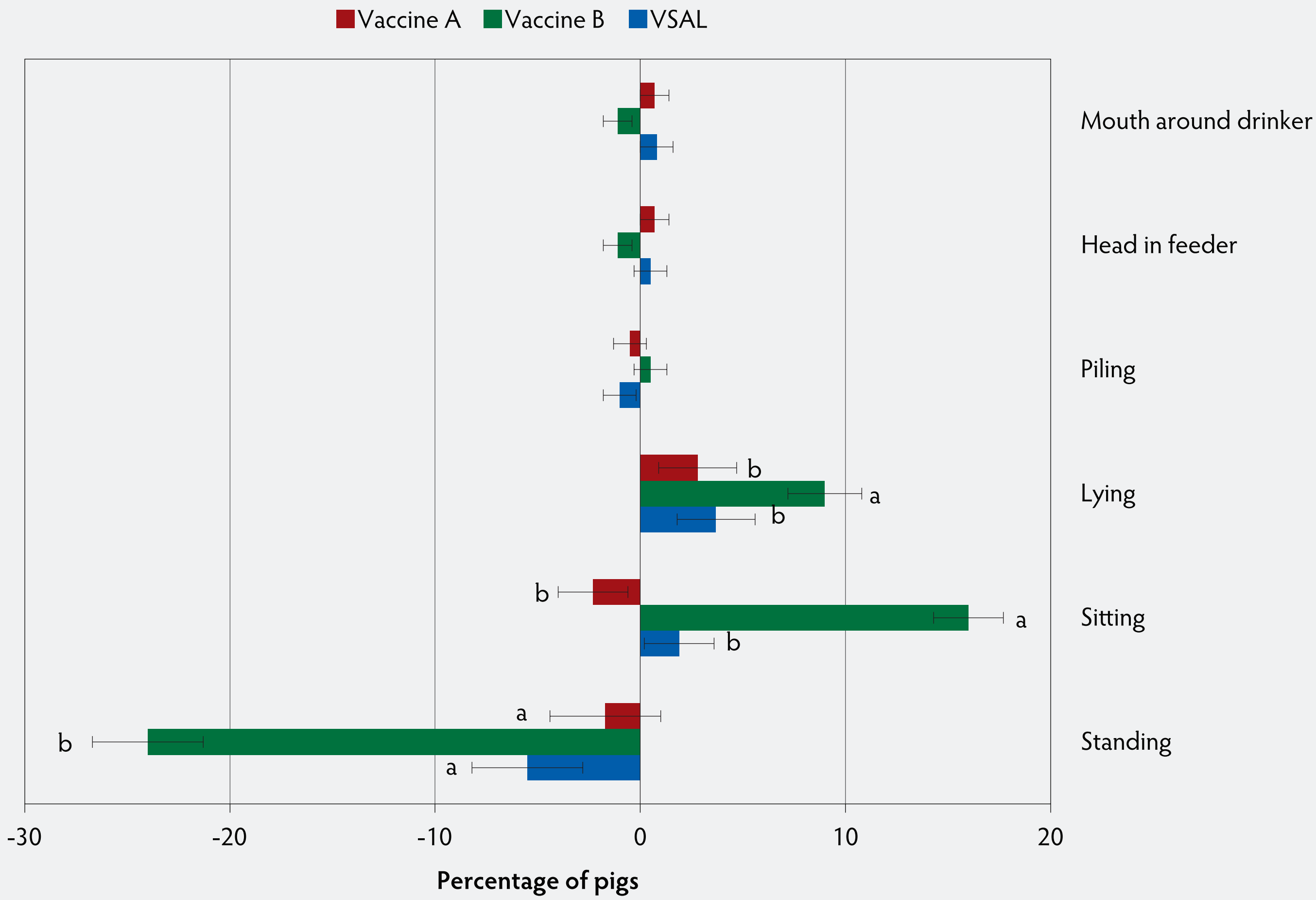


with more pigs lying approximately 6 hours after vaccination. In 2007, CircoFLEX, (Boehringer Ingelheim Vetmedica Inc, St Joseph, Missouri) a PCV2 subunit vaccine, was released.[9]

To quantify the transient behavioral differences noted in pigs after treatment with these vaccines, the authors chose the HAP.[10-13] All mammals display similar physiological alterations (ie, febrile response) and sickness behaviors (lethargy, decreased appetite and thirst, huddling, shivering, sleepiness, reduced grooming and exploration, uncoordinated body movements, and an increase in pain sensitivity)[14,15] to bacterial, viral, and protozoan pathogens. These alterations and sickness behaviors are derived from the energy cost diverted to the physiological response to an immunogen, subsequent antibody formation, and memory-cell development and nourishment.

The present study agrees with the injection effect on pig behavior, where a greater percentage of pigs within pens that received a vaccine injection were classified as Not Oriented 6 hours post injection. The purpose of the saline (VSAL) injection was to control for variation due to restraint handling and injection experience.[16,17] Additionally, due to the dosage timeline differences of Vaccine B being a two-stage vaccine and Vaccine A being a one-stage vaccine, Vaccine A pens of pigs received a saline injection, whereas Vaccine B pens of pigs received the Vaccine B injection on day 28 of age. Therefore, the post-vaccination differences may have been due to the vaccine complexities. Relatedly, pens of

Figure 4: Postural mean percentage (± SE) differences (calculated by subtracting the post-injection percentage of pigs from the pre-injection percentage of pigs) for commercial nursery pigs within each pen further delineated into Not Oriented posture and behavior categories (behaviors and postures described in Table 2) using a digital image methodology at the conclusion of the human approach paradigm (HAP; methodology described in Figure 2) pre- and post injection (vaccines and injection schedules described in Table 1). Means were compared within a behavior category. Means with no common superscript (ab) are significantly different (LS Means; $P < .05$).



pigs injected with Vaccine A may have not responded to the adjuvant at 6 hours and HAP reactivities may have increased after 6 hours. The increase in pig approachability post injection from VSAL-treated pens may be an indicator that the injection procedure itself is not a stressor. Conversely, pens of pigs treated with Vaccine B had the greatest average decrease in pigs classified as standing (24%) and the greatest average increase of pigs sitting (16%). The results indicate the Vaccine B vaccine may have induced a stronger innate immune response. However, regardless of vaccine, this study notes that the majority of pigs post injection assumed a lying posture. Few pens had pigs pre- and post injection that piled, which has been interpreted as a fear behavior.[18,19]

Fangman et al[11] used the HAP method to compare pre- and post injection nursery pig behavior within pens vaccinated with Ingelvac MycoFLEX (Boehringer Ingelheim Vetmedica Inc, St Joseph, Missouri) and Respisure-One (Pfizer Animal Health, Exton, Pennsylvania). Pens of pigs vaccinated with Respisure-One were less willing to approach the observer than pens of pigs vaccinated with Ingelvac MycoFLEX 6 hours post injection. Similarly, Bretey et al[12] measured pig latency to approach pre- and 6 hours post injection within the pen after vaccination with Ingelvac CircoFLEX (Boehringer Ingelheim Vetmedica Inc, St Joseph, Missouri), Ingelvac MycoFLEX, an Ingelvac CircoFLEX-Ingelvac MycoFLEX mixture (Vaccine A; (Boehringer Ingelheim Vetmedica Inc, St Joseph, Missouri), a Circumvent/M Plus Pac mixture (Merck, Kenilworth, New Jersey), and saline. The pens given CV-MP had the fewest pigs willing to approach the observer post injection (25.3 %) compared to pens injected with saline (9.6 %), Ingelvac MycoFLEX (12.3 %), and Ingelvac CircoFLEX (9.5%). Therefore, these studies suggest multi-dose exposures to vaccine adjuvants may elicit more rapid immune responses than do single-dose exposures.

There were no pre-injection behavior differences between treatment groups for average pen snout and tail-base distances from the observer. Post injection, average pig tail-base distances within each pen were closer to the human observer in the Vaccine A-treated pens than in the Vaccine B and VSAL treated pens. This result is difficult to interpret practically. Using tail and snout distances did not provide clear conclusions on any injection effects in nursery pigs. It is interesting to note that more anatomical locations were unobservable post injection.

Table 4: Behavioral pen mean percentages (± SE) of commercial nursery pig postures and behaviors when classified as Not Oriented in a human approach paradigm (HAP) pre- and post injection*

	Injection treatment			
	Vaccine A	Vaccine B	VSAL	
No. of pens	48	51	50	*P*
Pre-injection				
Standing†	81.2 ± 2.3	83.0 ± 2.2	86.3 ± 2.3	.28
Sitting	6.5 ± 1.2	3.8 ± 1.2	5.7 ± 1.2	.25
Piling	2.4 ± 0.7	1.4 ± 0.7	1.2 ± 0.7	.47
Lying	7.2 ± 1.7	8.5 ± 1.6	4.3 ± 1.6	.17
Head in feeder	1.6 ± 0.6	1.5 ± 0.5	1.2 ± 0.6	.90
Mouth around drinker	1.0 ± 0.5	1.9 ± 0.5	1.2 ± 0.5	.48
Post injection				
Standing	79.5 ± 2.8[a]	59.0 ± 2.7[b]	80.8 ± 2.7[a]	< .001
Sitting	4.2 ± 1.6[b]	20.4 ± 1.5[a]	7.7 ± 1.6[b]	< .001
Piling	1.4 ± 0.6	1.9 ± 0.5	0.8 ± 0.6	.34
Lying	10.9 ± 2.0[b]	17.5 ± 2.0[a]	7.1 ± 2.0[b]	< .01
Head in feeder	2.3 ± 0.6[a]	0.4 ± 0.6[b]	1.7 ± 0.6[ab]	< .10
Mouth around drinker	1.7 ± 0.5	0.8 ± 0.5	2.0 ± 0.5	.22

* Vaccines and schedules described in Table 1.
† Ethogram of behaviors and postures described in Table 2.
ab Means within a row with no common superscript are significantly different (LS Means; $P < .05$).

Possible explanations could be related to head position within the pen (more post-injection pigs holding their heads in a downward position). Another explanation is that pigs may have been closer together and thus anatomical locations were obstructed from view. Therefore, it is suggested that future work should include pig-to-pig distance and head position in relation to the body. In addition, it may be useful to conduct the HAP at additional time points after 6 hours post injection to determine when vaccination effects, if any, disappear. Additional work should include injection treatments at different production phases.

If this study were repeated, several additional measures could be included. First, a control group where pigs are not handled, as well as a group of pigs that are handled but not vaccinated, might be included in combination with injection treatments. This would help researchers more clearly identify the portion of the vaccination process (ie, pig handling, injection, or the immunogen) that may negatively impact pig behavior to the greatest degree. Secondly, the HAP would be conducted at later time points to determine when pigs return to baseline HAP values. This would help determine the length of time pigs exhibit lethargic behaviors post vaccination. In addition, physiological and performance measures such as serum cortisol, core body temperature, and feed intake would be useful to correlate with HAP to interpret the underlying mechanisms responsible for the altered behavior.

Implications

- Under the conditions of this study, pens of pigs orient less towards the human 6 hours after injection.
- Under the conditions of this study, when delineating post-injection behaviors and postures, vaccinated pens of pigs are categorized as displaying more lying behavior.
- Differences may exist in behavioral reactivities to vaccine injections.
- If the HAP were to be incorporated in an on-farm welfare assessment or auditing program, it would be important to know the age of the nursery pigs and the vaccine with which they are treated, and the protocol should be provided to accurately determine pig welfare.

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Conflict of interest

Dr Fangman was employed by Boehringer Ingelheim Vetmedica, Inc, during this study.

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Table 5: Commercial nursery pig snout and tail-base pen mean proximities (cm) from the index finger of a human observer during a human approach paradigm (HAP) within the behavior categories Touched, Oriented, and Not Oriented*

	Injection treatment			
	Vaccine A	Vaccine B	VSAL	
No. of pens	48	51	50	*P*
Pre-injection				
Touched, snout†‡	14.2 ± 2.5	14.3 ± 2.4	15.6 ± 2.3	.88
Touched, tail base	74.2 ± 2.2	73.9 ± 2.0	76.8 ± 2.2	.57
Oriented, snout	85.8 ± 2.3	86.9 ± 2.3	83.9 ± 2.3	.63
Oriented, tail base	116.4 ± 2.0	115.8 ± 2.0	114.5 ± 2.0	.80
Not Oriented, snout	119.8 ± 2.3	113.1 ± 2.4	116.4 ± 2.2	.13
Not Oriented, tail base	127.5 ± 1.5	125.7 ± 1.5	128.5 ± 1.4	.37
Post injection				
Touched, snout	28.8 ± 4.6	23.6 ± 6.4	18.2 ± 4.5	.26
Touched, tail base	70.5 ± 2.4	75.7 ± 3.4	72.3 ± 2.1	.44
Oriented, snout	83.8 ± 2.3[b]	91.8 ± 2.2[a]	87.7 ± 2.1[ab]	< .05
Oriented, tail base	110.9 ± 2.3[b]	121.1 ± 2.4[a]	118.4 ± 2.2[a]	< .01
Not Oriented, snout	121.5 ± 2.1	124.3 ± 2.0	124.8 ± 2.2	.49
Not Oriented, tail base	128.7 ± 1.3	127.0 ± 1.4	131.6 ± 1.4	< .10

* Vaccines and schedules described in Table 1.
† Ethogram of behaviors and postures described in Table 2.
‡ Snout anatomical measure was defined as the midpoint of the superior nose, and tail base was defined as the point of the pig's superior rear located at the tail base. Snout and tail-base proximities were measured using the ruler tool in Adobe Photoshop CS5 (Adobe Systems Inc, San Jose, California). In order to determine the actual distance in cm for snout distance, lengths collected from the digital image using the Adobe ruler were converted using the length of the nursery feeder (90.4 cm) and the feeder radius in pixels (620 pixels) for the digital image using the Adobe ruler tool. The conversion ratio was 6.9 (620 pixels ÷ 90.4 cm = 6.9).
ab Means within a row with no common superscript are significantly different (LS Means; $P < .05$).

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