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Original Research

Willingness-to-approach behavior of weaned pigs after injection with *Mycoplasma hyopneumoniae* vaccines

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Summary

Objective: To determine if field observations of reduced pig activity after injecting *Mycoplasma hyopneumoniae* vaccines could be confirmed and quantified by observing willingness to approach (WTA) an observer for 15 seconds.

Materials and methods: Three studies were conducted. Two weeks post nursery placement, pigs 17 to 23 days old were evaluated to determine WTA using the Swine Welfare Assurance Program behavioral protocol. Pigs were observed at 4:00 PM the day before injection and at 4:00 PM 24 hours later, 6 hours post injection. The difference between the two WTA scores is reported as a decrease in percent willing to approach. In Study One, 1832 pigs were vaccinated either with MycoFLEX® (36 pens) or RespiSure-ONE® (36 pens). In Study Two, 2568 pigs were injected with MycoFLEX® (39 pens), RespiSure-ONE® (39 pens), or saline (38 pens). In Study Three, 1750 pigs were injected with saline (35 pens) or not injected (35 pens).

Results: In Studies One and Two, the decrease in WTA was less for pigs vaccinated with MycoFLEX® than with RespiSure-One® (Study One, 11.2% versus 26.8%, $P < .001$; Study Two, 13.5% versus 35.8%, $P < .001$). In Study Two, the decrease in WTA did not differ between MycoFLEX® and saline-injected pigs ($P = .22$). In Study Three, the WTA did not differ between saline- and non-injected pigs (2.0% versus 6.1%, $P = .28$).

Implication: Willingness to approach a human in a nursery-pen environment may be a sensitive parameter for assessing vaccine reactivity 6 hours post vaccination.

Keywords: swine, approachability, willingness to approach, vaccine

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Resumen - Conducta de deseo de acercamiento de cerdos destetados después de la aplicación de vacunas de *Mycoplasma hyopneumoniae*

Objetivo: Determinar si las observaciones de campo de la reducción de actividad de los cerdos después de vacunarlos contra *Mycoplasma hyopneumoniae* podían confirmarse y cuantificarse al observar el deseo de acercamiento (WTA por sus siglas en inglés) a un observador por 15 segundos.

Materiales y métodos: Se condujeron tres estudios. Dos semanas después de arribar al área de destete, se evaluaron cerdos de entre 17 y 23 días de edad para determinar el WTA utilizando el protocolo de conducta del Programa de Aseguramiento de Bienestar Porcino. Los cerdos fueron observados a las 4:00 PM el día anterior a la inyección y a las 4:00 PM, 24 horas más tarde, 6 horas post inyección. La diferencia entre los dos puntajes de WTA se reportó como un decremento en el porcentaje de deseo de acercamiento. En el Estudio Uno, se vacunaron 1832 cerdos con MycoFLEX® (36 corrales) ó con RespiSure-ONE® (36 corrales). En el Estudio Dos, se inyectaron 2568 cerdos con MycoFLEX® (39 corrales), RespiSure-ONE® (39 corrales), ó solución salina (38 corrales). En el Estudio Tres, se inyectaron 1750 cerdos con solución salina (35 corrales) ó no se inyectaron (35 corrales).

Resultados: En los Estudios Uno y Dos, el decremento en WTA fue menor en los cerdos vacunados con MycoFLEX® que con RespiSure-One® (Estudio Uno, 11.2% contra 26.8%, $P < .001$; Estudio Dos, 13.5% contra 35.8%, $P < .001$). En el Estudio Dos, el decremento en WTA no difirió entre los cerdos inyectados con MycoFLEX® y solución salina ($P = .22$). En el Estudio Tres, el WTA no difirió entre cerdos inyectados con solución salina y los no inyectados (2.0% contra 6.1%, $P = .28$).

Implicación: El deseo de acercarse a un humano en un corral de destete puede ser un parámetro sensible para valorar la reactividad a una vacuna 6 horas post vacunación.

Résumé - Étude du comportement d'accord à approcher de porcs sevrés après injection de vaccins pour *Mycoplasma hyopneumoniae*

Objectif: Déterminer si les observations sur le terrain d'activités réduites des porcs après injection de vaccins pour *Mycoplasma hyopneumoniae* pouvaient être confirmées et quantifiées en observant l'accord à approcher (WTA) un observateur pour 15 secondes.

Matériels et méthodes: Trois études ont été réalisées. Deux semaines après l'entrée en pouponnière, des porcs âgés de 17 à 23 jours ont été évalués pour déterminer le WTA en utilisant le protocole de comportement du Programme d'assurance du bien-être porcin. Les porcs ont été observés à 16:00 heure le jour précédant l'injection et à la même heure 24 heures plus tard, soit 6 heures post-injection. La différence entre les deux pointages de WTA est rapportée comme une diminution en pourcentage du WTA. Lors de l'Expérience 1, 1832 porcs ont été vaccinés soit avec MycoFLEX® (36 enclos) ou RespiSure-ONE® (36 enclos). Lors de l'Expérience 2, 2568 porcs ont été injectés avec MycoFLEX® (39 enclos), ou de la saline (38 enclos). Lors de l'Expérience 3,

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1750 porcs ont été injectés avec de la saline (35 enclos) ou non injecté (35 enclos).

Résultats: Au cours des Expériences 1 et 2, la diminution du WTA était moindre pour les porcs vaccinés avec MycoFLEX® comparativement à ceux vaccinés avec RespiSure-ONE® (Expérience 1, 11.2% versus 26.8%, $P < .001$; Expérience 2, 13.5% versus 35.8%, $P < .001$). Au cours de l'Expérience 2, il n'y avait pas de différence dans la diminution du WTA entre les animaux vaccinés avec MycoFLEX® et les porcs injectés avec de la saline ($P = .22$). Lors de l'Expérience 3, il n'y avait pas de différence dans le WTA des animaux injectés avec de la saline et les animaux non-injectés (2.0% versus 6.1%, $P = .28$).

Implication: L'accord à approcher un humain dans l'environnement d'une pouponnière pourrait être un paramètre sensible pour évaluer la réactivité à un vaccin 6 heures post-vaccination.

Pigs consider the nursery environment in which they have resided for a few weeks to be their "environmental model," the place where they feel most comfortable and secure, and in turn their expectations are based on this place. Novel stimuli infrequently occur during the nursery phase. When a novel situation is implemented, this might result in innate survival responses by the individual pig.[1] How a pig behaves in response to novel environmental stimuli is based on cues received from the environment and processed by the pig using its basic sensory capabilities of vision, hearing, olfaction, and touch.[2-4] Some novel stimuli might be labeled as "more adverse" to the pig, for example, an injection procedure.[5,6] In the United States, pigs in the nursery phase receive vaccinations for circovirus, *Mycoplasma hyopneumoniae* (M hyo), *Erysipelothrix rhusiopathiae* (erysipelas), *Salmonella* serovars, and *Lawsonia intracellularis* (ileitis). These vaccinations are typically administered via intramuscular injection (circovirus, M hyo, and erysipelas) or drinking water (ileitis, *Salmonella*, and erysipelas). It has been noted by swine practitioners (oral communication, anonymous practitioner, 2008) that approximately 6 hours after intramuscular injection with some products, pigs lie down and become lethargic, and feed consumption is reduced. These alterations in the pigs' behavioral repertoire have been labeled by swine practitioners as the "buzz" response. In addition, producers and veterinarians have anecdotally noted that pigs become more reluctant, or less willing, to approach a caretaker. In these studies, we anticipated alterations in pig willingness-to-approach (WTA) behavior associated with the adverse environmental stimulation of the injection procedures. Therefore, the objectives of these studies were to determine if field observations of WTA behavior after injection with two different M hyo vaccines could be confirmed and quantified by observing post-vaccinal pig behavior changes for 15 seconds; to determine if field observations of WTA behavior after injection with either of two different M hyo vaccines and saline (control group) could be confirmed by observing post-injection pig behavior changes for 15 seconds; and to determine if field observations of WTA behavior of groups either injected with saline or not injected could be confirmed by observing post-injection pig behavior changes for 15 seconds.

Materials and methods

Animal care and husbandry protocols for these studies were overseen by the Boehringer Ingelheim Vetmedica Inc company veterinarian. These protocols were based on the US swine industry guidelines presented in the National Pork Board's Swine Care Handbook[7] and the PQA Plus Manual.[8]

Animals and location

Study One: Seventy-two pens of weaned pigs, housing approximately 25 pigs per pen, for a total of 1832 crossbred pigs (GPK35 × EB5, Monsanto Choice Genetics, St Louis, Missouri) 17 to 23 days of age were obtained from a production system negative for porcine reproductive and respiratory syndrome (PRRS) and swine influenza virus, and positive for M hyo. The study was conducted during 4 consecutive days in October 2007 at a commercial mechanically ventilated nursery facility in South Central Missouri.

Study Two: One hundred and sixteen pens of weaned pigs, housing approximately 22 pigs per pen, for a total of 2568 crossbred pigs (Newsham Females, West Des Moines, Iowa × Danbred boars, Columbus, Nebraska) 17 to 23 days of age were obtained from a production system negative for PRRS and swine influenza virus, and positive for M hyo. The study was conducted during 4 consecutive days in December 2007 at a commercial mechanically ventilated nursery facility in Central Iowa.

Study Three: Seventy pens of weaned pigs, housing 25 pigs per pen, for a total of 1750 crossbred pigs (GPK35 × EB5, Monsanto Choice Genetics) 17 to 23 days of age were obtained from a production system negative for PRRS and swine influenza virus and positive for M hyo. The study was conducted during 4 consecutive days in March 2008 at a commercial mechanically ventilated nursery facility in South Central Missouri.

Diets, housing, and husbandry

Studies One and Three were conducted in similar facilities within the same production system located in South Central Missouri. Study Two was conducted in a different production system with facilities very similar to those in Studies One and Three. In all three studies, the pigs were separated by sex (opposite sides of the barn). Each 1.8-m × 3.0-m nursery pen provided 0.21 and 0.23 m^2 per pig for males and females, respectively. There were no solid dividers between pens; steel dividers were 3.0 m length × 78.7 cm height. Woven wire flooring (3-gauge, Boss Hog; J & L Wire, St Paul, Minnesota) was utilized in all pens. Pigs had ad libitum access to a pelleted diet (3413 kcal per kg metabolizable energy [ME] and 24% crude protein [CP]) formulated to meet requirements.[9] Diets were provided in a 10-hole dry feeder (14.0 cm depth × 10.2 cm height × 91.4 cm length; Automated Production Systems, Assumption, Illinois) with a pelleted-feed capacity of 65 kg. Each pen contained one stainless steel nipple cup drinker (12.7 cm depth × 25.4 cm height × 16.5 cm width; Farmweld, Teutopolis, Illinois). Incandescent lights were turned on for 1 hour at 7:00 AM and again for 1 hour at 4:00 PM daily during caretaker observation periods, providing a total of 10 hours of either natural or supplemental light in the pig space each day and 14 hours of darkness, respectively.

Treatments and experimental design

In all studies, pigs were identified by body weight (subjectively scored by the site manager and field staff, with each pig placed into either a light, medium, or heavy category) and sex (barrows and gilts), and then were assigned to pens so that pen weight and sex were even across pens (according to best management practices). These pigs were not weighed, and, for these variables, the data were not blocked for analysis. Treatment groups were then assigned to pens in an alternating manner. The experimental unit was the pen (containing the individual

nursery pigs). All treatments were assigned within rooms within the same barn. Day 0 was defined as the day in which the pigs were assigned to treatment group and those that were injected received the intramuscular injection of vaccine or saline.

Study One: Two treatments administered at 5 weeks of age were compared. The first treatment, MycoFLEX (n = 36 pens), was defined as a single intramuscular (IM) dose of 1 mL Ingelvac MycoFLEX vaccine (Boehringer Ingelheim Vetmedica Inc, St Joseph, Missouri). The second treatment, Resp1 (n = 36 pens), was defined as a single IM dose of 2 mL RespiSure-One vaccine (Pfizer Animal Health, New York, New York). Pens were alternately vaccinated with one of the two vaccine treatments. This study purposely lacked the inclusion of a placebo-injected group to quantify responses attributable solely to the injection procedures, as this study was conducted in the pig flow of a commercial production system and it was not economically feasible to consider a nonimmunized portion of this population in Study One.

Study Two: Three treatments administered at 5 weeks of age were compared. Treatments were MycoFLEX, (n = 39; approximately 22 pigs per pen), defined as a single IM dose of 1 mL Ingelvac MycoFLEX vaccine, and Resp1 (n = 39; approximately 22 pigs per pen), defined as a single IM dose of 2 mL RespiSure-One vaccine; and Saline (n = 38; approximately 22 pigs per pen), defined as a single IM dose of 1 mL phosphate buffered saline. Pens were alternately injected with one of the two vaccines or saline. In an effort to address the shortcomings of Study One, Study Two included a placebo-injected group to quantify responses attributable solely to the injection procedures.

Study Three: Two treatments administered at 5 weeks of age were compared. Treatments were Saline, (n = 35; approximately 26 pigs per pen), defined as a single IM dose of 1 mL phosphate buffered saline; and Non-treated (n = 35; approximately 26 pigs per pen), defined as no injections or treatments. There was no interaction between the non-injected pigs and a technician and there was no crowding of non-injected pigs on Day 0 (ie, the Non-treated pigs were not crowded to the alley end of the pen as were the pigs that were injected with saline). Pens were alternately treated with saline or not treated. Study Three was conducted to quantify responses attributable to pigs interacting with people on two occasions with no physical contact or interventions.

Injection technique

In all three studies, injections were made into the lateral cervical musculature on the right side of the neck using a 16-gauge, 5/8″needle. Within a study, the same technicians (technician defined as the person who held the syringe and had contact with the pigs when administering the injection) performed injection procedures for all treatments: two technicians in Studies One and Two and a single technician in Study Three. Pigs were moved by a sort board towards the alley end of their home pen. Pigs were not picked up and individually handled, in an effort to avoid any additional handling-associated stressors on the pigs.[10-13] The technicians visually identified and selected a pig among the crowded pigs and in 1 second inserted the needle into the neck and administered the preset dose of the vaccine from an automatic syringe (Studies One and Three, Uni-Matic 2-mL multi-dose syringe, Air-Tite Products Co, Inc, Virginia Beach, Virginia; Study Two, Felton 2-mL multi-dose syringe, Model ST2VU, Felton Medical Inc, Lenexa, Kansas). A mark was then placed between the scapulas of each pig using an animal-safe crayon (Raidex Animal Marking Sticks; Thousand Hill Supply, Walworth, New York) to avoid injecting the same pig twice.

Willingness-to-approach methodology

In all three studies, the pigs were observed 2 weeks post nursery placement (Study One, October 16, 2007; Study Two, December 26, 2007; and Study Three, March 27, 2008), when individual pigs were evaluated to determine their willingness to approach an observer. The WTA behavioral observations were conducted in all treatment groups on Day -1 and Day 0. Pre-injection baseline WTA observations were conducted at 4:00 PM on Day -1. Post-injection observations were conducted on Day 0 after injections were administered. Injection procedures began at 7:00 AM and were completed by 10:00 AM, and the 6 hours post-injection WTA observation took place at 4:00 PM, 24 hours after the pre-injection evaluation. The difference between these two values is reported here as a decrease in percent willing to approach.

Observation procedures were consistent with the animal observation procedures defined in the Swine Welfare Assurance Program (2003) behavior protocol (publication no longer available). Two different observers conducted the WTA methodology ("observer" defined as having no previous contact with these pigs). In Studies One and Three, one observer made all baseline WTA observations and a different observer made all post-injection WTA observations. In Study Two, the same observer conducted both the baseline and post-injection observations. This methodology was utilized in Studies One and Three to eliminate any familiarity that the pigs may have developed with the baseline observer and thus avoid any positive behavioral bias that may be associated with this observer familiarity.[14,15] In Study Two, the availability of labor limited the number of observers taking part in this study, and therefore only one observer conducted the observations on both days. All observers were blinded to treatment, wore individual sets of clothing and boots that were similar in design and color, and wore no artificial scents.

Each observer carried a pen, a clipboard containing one sheet of paper that had the floor-plan diagram of the barn layout, and the pre-recorded pen inventory. Willingness-to-approach observations began after the observer quietly entered a pen by stepping over the gating that separated the alleyway from the pen and then immediately crouched down in front of the alley-way gate and extended and held still one leather-gloved hand in the direction of the pigs.[4,15,16] The observer observed the stop watch and avoided eye contact with the pigs during the 15-second period.[5,17] At the conclusion of the 15-second period, the observer raised his or her head and scanned the pen to record the number of pigs interacting with the observer (defined as pig-to-human contact) and the number of pigs facing the observer in the semi-circle (Figure 1: a female observer is pictured, in Study Three; in Studies One and Two, male observers conducted the evaluations). Also recorded were the numbers of pigs that took one step toward the observer from any location within the pen, assuming a stance such that both eyes of that pig could be seen. These additional pigs may have been unable to enter the described semi-circle of pigs due to space availability within the pen. Pigs not fulfilling any of these criteria were classified as "unwilling to approach" the observer.

After the observer had completed the WTA scan for that pen, he or she proceeded to walk into subsequent pens in a side-to-side fashion, ie, pigs in a pen adjoining an observed pen were not the next pigs observed.

Willingness-to-approach calculations

To calculate the percentage of pigs willing to approach and differences between Day -1 and Day 0, three equations were utilized. First, percentage of pigs willing to approach Day -1 = total pigs that approached observer Day -1 in a pen ÷ total number of pigs in that pen. Second, percentage of pigs willing to approach Day 0 (post injection) = total pigs that approached observer Day 0 (post-injection) in a pen ÷ total number of pigs in that pen. Third, the decrease in percent willing to approach in each pen was the difference in WTA (%) between Day -1 and Day 0 = WTA% Day -1 – WTA% Day 0.

Statistical analysis

Data from all three studies were analyzed via paired *t* test to determine the differences in the WTA observations within treatment groups. Two-sample *t* tests were utilized in Studies One and Three, while one-way ANOVA was utilized in Study Two to determine the differences in the WTA observations between treatment groups.

Since the WTA measurements in each study were of normal distribution and standard deviation, they were analyzed as continuous responses within each study. Pen was the experimental unit for all studies. Within treatment, paired *t* tests were conducted to determine whether the difference in WTA from pre-injection to 6 hours post-injection was greater than what could happen by chance (for all studies). Two-sample *t* test (Studies One and Three) and one-way ANOVA (Study Two) models were used to compare the percentages of pigs approaching (per pen) between treatments. Tukey's HSD was used to discern differences among treatments in the ANOVA analysis. Results were considered significant when $P < .05$.

Results

During the observation periods, some pigs in an adjoining pen demonstrated an interest in the WTA activities. Of interest, the pigs in adjoining pens never came into direct contact with the observer.

Figure 1: Observing the willingness-to-approach (WTA) behavior of nursery-aged pigs either 24 hours before or 6 hours after they had either been injected with saline or not injected (no treatment or physical contact). At the conclusion of a 15-second period, the observer raised her head and scanned the pen to record the number of pigs interacting with the observer (defined as pig-to-human contact) and the number facing the observer in the semi-circle (Study Three).

Study One: Pre-injection WTA percentages did not differ between the two treatment groups (Table 1). For both treatment groups, the WTA percentage was lower post injection (Day 0). However, the decrease in percent WTA from Day -1 to Day 0 was greater for the Resp1 group than for the MycoFLEX group (Table 1).

Study Two: Pre-injection WTA percentages did not differ among the three treatment groups (Table 2). For all treatment groups, WTA percentage decreased post injection and these differences were significant (Table 2). However, the decrease in percent WTA from Day -1 to Day 0 was greater for the pigs that received Resp1 than for the MycoFLEX and Saline groups (Table 2). Change in percent WTA did not differ between the MyoFLEX and Saline groups ($P = 22$).

Study Three: Neither pre-injection nor post-injection WTA percentages differed significantly between the two treatment groups (Table 3). For both treatment groups, the WTA percentage decreased numerically but not significantly between Day -1 and Day 0, and there was therefore no significant difference between the two treatments in the change from Day -1 to Day 0 (Table 3). Percent WTA differed significantly between Day -1 and Day 0 for the Non-treated group, but not for the Saline group (Table 3).

Discussion

Previous work has demonstrated the important relationship that exists between the caretaker and the pig, and how behavior, physiology, and performance can be altered when a novel stimulus is introduced into the pigs' environment.[2,12,13,17] It is important for those involved in swine well-being to consider reliable and practical tools that could be implemented in commercial practice to assess reactivity between a human and pig. Several studies have addressed this concept and have used a 15-second period of time, defined as "latency for individual animals to approach an observer."[4,5,14,15,18] However, it maybe useful to redefine this "latency to approach" terminology as a variable time response to "willingness to approach" terminology that can be defined as a fixed variable of time. When the time required to observe the pigs is fixed, this technique can be readily adapted to a modern production system and utilized to observe and record behavior changes in pigs after an intervention (eg, injection procedure) or environmental change (eg, temperature fluctuation).

The intended purpose of Study One was to determine if field observations of WTA after injection with two different M hyo vaccines could be confirmed and quantified by

observing postvaccinal pig behavior changes for 15-second observation periods.

At 6 hours post injection, an observer blinded to treatments was able to utilize the WTA evaluation technique to quantify the change in WTA. In Study One, the Resp1 treatment group demonstrated a significant reduction in the percentage of pigs willing to approach the observer 6 hours after injection of a vaccine. This was the first attempt to apply the WTA methodology, and it was encouraging to note the apparent value of this technique to determine WTA differences. However, this first study notably lacked the inclusion of a saline control group, and a second study was conducted to correct this deficiency.

The intended purpose of Study Two was to collect additional field observations of WTA after injection with two different M hyo vaccines and to compare these observations to those of a saline control group. The second study included the same two M hyo vaccine treatment groups used in Study One, with the notable addition of a saline control group to assess the WTA changes associated with the injection procedure alone (independent of a vaccine effect).

The Study Two results may suggest that some of the reduction in WTA after injection is a consequence of a negative pig-human interaction associated with placing the 16-gauge, 5/8-inch needle in the lateral cervical musculature on the right side of the neck. The additional decrease in WTA is believed to be associated with the characteristics of the vaccines. Study Two demonstrates the value and robust nature of the WTA method to determine differences and similarities in WTA between vaccine and saline control groups.

Possible explanations for the observed differences between vaccine groups in their WTA responses after injection include the dosing volume differences between Resp1 and MycoFLEX, adjuvant formulations, differences in manufacturing between the two products, and differences in pig immune responses (cytokines or interleukins).

Study Two differed from Studies One and Three in that the same person made observations on both the Day -1 and Day 0 in Study Two, while two different observers were used in Studies One and Three. Study Two also utilized a Felton 2-mL multi-dosing syringe, while a Uni-matic 2-mL multi-dosing syringe was used in Studies One and Three. These differences in Study Two did not result in any observed differences in comparable outcomes.

Table 1: Percentages of 5-week-old nursery pigs that showed willingness to approach (WTA) a human observer pre-injection with *Mycoplasma hyopneumoniae* vaccine (Day -1) and 24 hours later, 6 hours post injection (Day 0) (Study One)*

	Treatment				
	MycoFLEX		Resp1		
Parameter	Mean	SEM	Mean	SEM	*P*†
No. of pens	36	NA	36	NA	NA
Average no. of pigs/pen	25.4	0.31	25.5	0.29	.85
WTA (%)					
Day -1‡	60.86	0.02	66.03	0.02	.13
Day 0§	49.69[a]	0.03	39.17[b]	0.03	.02
Δ (Day -1 to Day 0)¶	11.17[a]	0.03	26.86[b]	0.03	< .001
*P***	< .001	NA	< .001	NA	NA

* Pigs were treated either with MycoFLEX (1-mL dose; Boehringer Ingelheim Vetmedica, Inc, St Joseph, Missouri; MycoFLEX) or with RespiSure-One (2-mL dose; Pfizer Animal Health, New York, New York; Resp1), each administered as a single intramuscular dose injected into the right lateral cervical musculature using a 16-gauge needle. The same two technicians performed injection procedures for both treatments. Pigs were observed for percent WTA during a 15-second period when an observer, blinded to treatment, entered the pen the day before injection (Day -1) and 24 hours later, 6 hours after injection (Day 0). Different observers made the Day -1 and Day 0 WTA observations.

† Between treatments, paired *t* tests were conducted to determine whether the change in percent WTA between Day -1 and Day 0 was statistically different ($P < .05$).

‡ Percentage of pigs willing to approach the observer Day -1 (pre-injection) = (number of pigs that approached observer in a pen) ÷ (total number of pigs in that pen).

§ Percentage of pigs willing to approach the observer Day 0 (post injection) = (number of pigs that approached observer in a pen) ÷ (total number of pigs in that pen).

¶ Difference between pre- and post-injection percent WTA = (percent WTA Day -1 in a pen) - (percent WTA Day 0 in that pen).

** Within treatment, paired *t* tests were conducted to determine whether the change in percent WTA between Day -1 and Day 0 was statistically significant ($P < .05$)

ab Means within a row with no common superscript are significantly different ($P < .05$).

SEM = standard error of the individual mean; NA = not applicable.

A third study was conducted to determine if WTA response differences could be detected when a saline control group was compared to a non-treated group by observing post-injection pig WTA changes for 15 seconds. In Study Three, there were no differences between Saline and Non-treated groups. The Saline group did not differ in WTA between Day -1 and Day 0. However, the Non-treated pigs did differ in percent WTA between Day -1 and Day 0. This finding is surprising, as it could be hypothesized that no differences between Day -1 and Day 0 percent WTA would be observed in pigs that received no injections. This difference may be explained by the slightly higher baseline pre-injection percent WTA noted in the Non-treated group, as the post-injection WTA percent was nearly identical in the two treatment groups.

In Study Three, female observers conducted the evaluations on Day -1 and Day 0, while male observers were used in Studies One and Two. This difference in Study Three was not associated with any observed differences in comparable outcomes.

It is important to note that factors other than the vaccine administration may have influenced WTA.

It appears that this method of evaluating nursery pigs that are willing to approach an observer following an injection can determine differences in the reactivity of M hyo vaccines administered to pigs at 5 weeks of age. From the results of Study Two, it would also appear that nursery pigs demonstrate a decrease in their willingness to approach an observer after an injection of saline, while in Study Three there was no difference between the WTA responses of pigs treated with saline or not treated. Nursery pigs may also

Table 2: Percentages of 5-week-old nursery pigs that showed willingness to approach (WTA) a human observer pre-injection with *Mycoplasma hyopneumoniae* vaccine or saline (Day -1) and 24 hours later, 6 hours post injection (Day 0) (Study Two)*

	Treatment						
	Saline		MycoFLEX		Resp1		
Parameter	Mean	SEM	Mean	SEM	Mean	SEM	*P*†
No. of pens	38	NA	39	NA	39	NA	NA
Average no. pigs per pen	21.5	0.81	22.7	0.87	22.2	0.61	.53
WTA (%)							
Day -1‡	46.89	0.04	49.74	0.03	49.54	0.04	.82
Day 0§	38.53[a]	0.04	36.28[a]	0.04	13.67[b]	0.02	< .001
Δ (Day -1 to Day 0)¶	8.36[a]	0.02	13.46[a]	0.03	35.87[b]	0.03	< .001
*P***	< .001	NA	< .001	NA	.001	NA	NA

* Pigs were treated either with MycoFLEX (1-mL dose; Boehringer Ingelheim Vetmedica, Inc, St Joseph, Missouri; Myoflex), with RespiSure-One (2-mL dose; Pfizer Animal Health, New York, New York; Resp1), or with phosphate buffererd saline (1-mL dose; Saline), each administered as a single intramuscular dose injected into the right lateral cervical musculature using a 16-gauge needle. The same two technicians performed all injections. Pigs were observed for percent WTA during a 15-second period when a single observer, blinded to treatment, entered the pen the day before injection (Day -1) and again 24 hours later, 6 hours after injection (Day 0).

† One-way ANOVA to determine whether parameters were statistically different among treatment groups ($P < .05$).

‡ Percentage of pigs willing to approach the observer Day -1 (pre-injection) = (number of pigs that approached observer in a pen) ÷ (total number of pigs in that pen).

§ Percentage of pigs willing to approach the observer Day 0 (post injection) = (number of pigs that approached observer in a pen) ÷ (total number of pigs in that pen).

¶ Difference between pre- and post-injection percent WTA = (percent WTA Day -1 in a pen) - (percent WTA Day 0 in that pen).

** Within treatment, paired *t* tests were conducted to determine whether the change in percent WTA between Day -1 and Day 0 was statistically different ($P < .05$).

ab Means within a row with no common superscript differ (Tukey HSD; $P < .05$).

SEM = standard error of the individual mean; NA = not applicable.

demonstrate a slight decrease in their willingness to approach an observer even when there is no treatment between observations. However, the WTA-evaluation technique may be better suited to differentiate nursery pigs approaching an observer when a vaccine is administered than groups of pigs receiving only a saline injection or no treatment at all.

This WTA-evaluation technique is subject to variability associated with the nursery pig's previous interactions with humans at this site. However, the pigs evaluated in these studies were immediately willing to approach the observers, indicating that previous interactions between pigs and caretakers had been positive (baseline percent of pigs willing to approach ranged from 46.9% to 66.0%). Future studies that include this WTA-evaluation technique should consider this variability when determining the number of pens to include in each treatment group. The authors suggest that a minimum of 36 pens per treatment may be required to account for behavior variability when determining WTA and to allocate to treatment on the basis of baseline measures.

Future studies will be needed to define the ethogram of the remaining pigs not counted in the WTA observations. The authors are fully aware that this is a novel approach to assessing swine WTA after injection, and we recognize the need for additional research in this area. Immediate areas of consideration include, but are not limited to, the genetics of the pig, housing designs, space allowance, and age of the pig being studied. In addition, further validation of the consistency of observations between multiple observers (inter-reliability) and within an observer (intra-reliability) must be conducted. Finally, combining the WTA methodology with other aspects of predicting swine well-being would be advantageous. For example, physiological aspects of the behavioral changes observed will include measures of interleukin and cytokine changes, measure of febrile response, or both.

Implications

- An adaptation of the National Pork Board's 2003 Swine Welfare Assurance Program swine-behavior protocol can be modified to quantify behavior changes in 5-week-old pigs after injection with commercial M hyo vaccines and is defined here as willingness to approach (WTA) an observer within a 15-second period.
- Differences may exist in the comparative reactivities of injectable vaccines.
- Under the conditions of this study, WTA decreases more after pigs have been vaccinated with Resp1 than with MycoFLEX.
- WTA decreases in nursery pigs when they receive a saline injection.
- WTA may decrease in nursery pigs when no treatment intervention is applied.
- WTA may be a sensitive parameter for assessing vaccine reactivity in nursery pigs 6 hours post vaccination.

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Table 3: Percentages of 5-week-old nursery pigs that showed willingness to approach (WTA) a human observer pre-injection with saline or no treatment (Day -1) and 24 hours later, 6 hours post injection (Day 0) (Study Three)*

	Treatments				
	Saline		Non-treated		
	Mean	SEM	Mean	SEM	*P*†
No. pens	35	NA	35	NA	NA
Average no. pigs per pen	26.1	0.47	27.0	0.46	.21
WTA (%)					
Day -1‡	53.42	0.02	57.61	0.02	.15
Day 0§	51.41	0.02	51.53	0.03	.97
Δ (Day -1 to Day 0) ¶	2.01	0.03	6.08	0.02	.28
*P***	.49	NA	.02	NA	NA

* Pigs were treated either with a 1-mL dose of phosphate buffered saline injected into the intramuscular lateral cervical musculature on the right side of the neck using a 16-gauge needle (Saline) or were not treated (Non-treated). The technician performing the injections did not interact with the Non-treated pigs in any way. Pigs were observed for percent WTA during a 15-second period when an observer, blinded to treatment, entered the pen the day before injection (Day -1) and 24 hours later, 6 hours after injection (Day 0). Different observers made the Day -1 and Day 0 WTA observations.

† Paired *t* tests were conducted to determine whether parameters were statistically different between treatment groups ($P < .05$).

‡ Percentage of pigs willing to approach the observer Day -1 (pre-injection) = (number of pigs that approached observer in a pen) ÷ (total number of pigs in that pen).

§ Percentage of pigs willing to approach the observer Day 0 (post injection) = (number of pigs that approached observer in a pen) ÷ (total number of pigs in that pen).

¶ Difference between pre- and post-injection percent WTA = (percent WTA Day -1) - (percent WTA Day 0 in that pen).

** Within treatment, paired *t* tests were conducted to determine whether the change in percent WTA between Day -1 and Day 0 was statistically different ($P < .05$).

SEM = standard error of the individual mean; NA = not applicable

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