Journal of Visualized Experiments

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--Manuscript Draft--

Article Type:	Invited Methods Article - Author Produced Video
Manuscript Number:	JoVE62099R2
Full Title:	Transition of Farm Pigs to Rresearch Pigs Using a Designated Checklist Followed by Initiation of Clicker Training – a Refinement Initiative
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TITLE:

Transition of Farm Pigs to Research Pigs Using a Designated Checklist Followed by Initiation of Clicker Training – a Refinement Initiative

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KEYWORDS:

Pigs, Swine, 3Rs, Refinement, Training, Socialization, Habituation, Acclimatization

SUMMARY:

Refinement of porcine studies is achieved by introducing a standardized checklist and positive reinforcement training using a clicker. This work supports the collection of samples and the conduct of daily chores related to the animals.

ABSTRACT:

Implementation of 3R initiatives is important. To refine the life of laboratory pigs, we focused on the time period prior to inclusion of animals in experiments. We improved the checklist during the development. By using the checklist each individual pig's progress was documented. This aids the caretakers so they remain focused on the actions necessary to socialize the animals and limit the animal's stress and anxiety level. During this phase, the pigs become calm and ready to commence additional clicker training to further limit stress during the experimental period. The pigs will readily allow basic outcome measures to be collected without sedation or fixation.

The protocol is divided in two. First the transition phase that begins as the pigs arrive at the research facility. Introducing treats and gradually closer contact to the caretakers are the first steps. Then, the caretaker introduces actions to be able to touch the head and hind, to mimic e.g. manipulating the ear to take a blood sample or the hind to obtain a rectal temperature. A strong foundation is established in the animal-caretaker relationship and this swiftly minimizes the stress the pigs express after arrival. Secondly, the positive reinforcement training commences to teach the animal how to carry out more complex tasks without becoming stressed. A clicker is introduced as a secondary reinforcer.

Time, structure, education and thorough communication seem to be the most important criteria to succeed in the application of this protocol. In addition to having calm and compliant pigs, the

caretakers report that they enjoy the focus they are allowed to give each individual pig, adding to their overall job satisfaction. Having caretakers with a strong commitment, and a focus on the transition progress and welfare of each pig, additionally strengthens the department's Culture of Care.

INTRODUCTION:

The principles of the 3Rs (Replacement, Reduction and Refinement) discussed by Russell and Burch in 1959, form today's foundation for high ethical standards concerning the use of animals for experimental purposes. In biomedical research facilities engaged in preclinical laboratory animal studies, great importance must be adhered to the refinement principle. "Refinement means any decrease in the incidence or severity of inhumane procedures applied to those animals which still have to be used"[1].

A contemporary definition additionally states that refinement is "advancing research into animal welfare by exploiting the latest in vivo technologies and by improving understanding of the impact of welfare on scientific outcomes"[2]. This implies that not only are today's refinement initiatives important for the welfare of the animal per se, but that they are also important to research quality as scientific outcomes will benefit from these initiatives [3].

One refinement initiative to consider is to socialize and train experimental animals. They may be trained to perform a certain task (e.g., step onto a scale or behave calmly) while blood samples are drawn. Some species may be more naturally disposed to training than others and this impacts the tradition for training laboratory animal species. Traditionally, training of dogs has been performed for centuries (e.g., in order to use the dog in hunting). These historic traditions have most certainly made the training of dogs in the biomedical research setting more straightforward. Together with non-human primates, training of laboratory dogs has been discussed and undertaken for quite some time[4,5].

Common to both of these species is that they rank high on the "socio-zoological scale". This scale ranks animals according to their place and use in human society [6]. Ranking high also carries with it a large public demand to treat the animals as humanely as possible and limit their stress and anxiety level. Rodents, in contrast to dogs and non-human primates, are placed in the lower end of the socio-zoological scale. They have not received equivalent public attention and care. Historically they have been treated less well - presumably also due to their ability to spread life-threatening disease. However, during the last few years the training of rodents has been implemented in preclinical research settings and hereof related scientific benefits have not gone unnoticed. Both rats and mice are interestingly quite easily trained and the training also limits *their* stress-response and thus strengthens scientific outcomes[7,8].

Reporting on how to socialize and train pigs in a laboratory animal setting is to our knowledge scarce[9]. On the socio-zoological scale, pigs are placed in-between dogs and rodents. We recognize the species primarily as a food resource, but nevertheless quite a large number of pigs are also used in biomedical research. In the EU alone, approximately 75,000 pigs were used annually for scientific purposes in 2015-2017 [10]. This number encompasses both farm pigs and

miniature pigs. There is an increasing focus on refining the lives of these animals by training as a measure to comply with the 3Rs. Both farm pigs and miniature pigs can indeed be trained [11,12], but their starting point in terms of socialization varies. Miniature pigs are bred for research and socialized during their early life span. They are bred to have a calm temper. Farm pigs are bred for consumption and often not socialized at all. Finisher pigs for meat production in Denmark are typically housed in high density groups of 15-25 pigs where they have to compete to earn a position in the herd's hierarchy. Their temper reflects that ability. Often, they are housed in stable facilities with a partially slated floor to limit the workload of mucking out. To optimize weight gain, they are fed ad libitum through automated food and water delivering systems. Hence, their contact with farm workers is limited and they will become stressed when handled. This poses certain challenges when the animals are transferred to a research facility. Here the animal welfare, the husbandry, and the 3Rs play a key role[13,14]. From a research perspective, each individual animal is important to obtaining the best possible research results. The welfare of the animals is important to ensure a low inter-animal variation in regard to stress, anxiety and fear. For these reasons it is important to pay attention to the transition from a "herd animal" to an "individual". Upon arrival, the pigs enter a new environment with new scents, new food, day-to-day contact with animal caretakers and often also new pen mates. To a pig, the shift can be very stressful and the importance of an acclimatization period of several days is acknowledged in laboratory animal science[15].

By structuring the acclimatization period, we have created what we call a "transition phase" using a structured step-by-step checklist. The checklist ensures that each pig receives sufficient caretaker contact and learns to allow basic handling for examination and sample collection without being stressed. Continuous work can then be carried out to train the pigs using positive reinforcement training targeted to each specific research project[16,17]. A clicker is used to signal a food reward when the desired action is performed. Combining the steps from the transition phase with additional clicker training of the pigs gives the animals a smooth settling in at the research facility. They become calm and easy to handle and can, with little effort, be taught to stand still for e.g. blood sample collection. By the use of this type of handling protocol, old-fashioned techniques such as sedation of the animals or snares are replaced. To make sure all pigs are handled sufficiently a checklist is filled out.

It is becoming widely accepted that animals do indeed feel positive emotions and so focus when working with the animals should not solely be on the removal of negative emotions [18]. The training session with the caretaker is an example of a positive emotional experience. It is quite enjoyable for the pig as it receives treats and attention. Besides the benefit of reducing handling-stress and fear related behaviors at the facility, training the animals provides an opportunity to give the animals positive emotional experiences. The adding of positive experiences may counteract some of the negative experiences that laboratory animals encounter in relation to experiments performed and will thus further increase animal welfare [19,20].

While the pigs evidently enjoy the training sessions, the sessions simultaneously offer the caretaker an opportunity to strengthen the bond to each individual pig. Being an animal caretaker performing painful actions such as e.g. injections of the animals is part of the job. However, being

able to train the animals and turn these actions into positive experiences is rewarding in itself. It increases the commitment of the caretakers and plays a key role in strengthening the department's culture of care[21-23].

The pigs were housed according to the Institution's Animal Welfare Policy, which refers to national legislation on animal experimentation, housing and husbandry. The protocol can be applied to weaned pigs of any age. For the development of the transition checklist (**Figure 1**) a total of 40 female crossbred farm pigs (Danish Landrace-Yorkshire-Duroc) weighing 35-80 kg were used (**Table 1**). The pigs were allocated to a group dependent on their arrival time. The pigs were housed in the research stable facility in the period January – September 2020. All farm pigs are bred on a traditional sow farm and moved to a collaborating farm when weighing approximately 15 kg. At least two weeks before inclusion in survival studies, the pigs are transported to the university's research stable facility. During the first two weeks the transition from farm pig to research animal takes place. The protocol is composed of non-harmful and positive actions and hence may be initiated immediately as part of the acclimatization period.

The pigs were housed one by one in pens measuring 3.4 - 6.8 m^2 on solid concrete floors and have access to water via an automatic water supply. There is a feeding trough for each pig and at least one section of the separation to the neighboring pens allows snout contact. When pigs are scheduled to receive surgical interventions or have equipment implanted, they are housed alone to avoid pen mates licking and biting in wounds and pulling implants out. The bedding material is straw and the animals are further enriched with hay and different activity toys e.g. rope, balls, buckets, chew sticks made from plastic (**Figure 2**).

The animals come from a herd included in the national health-monitoring program for farm pigs, which means they are screened for seven pathogens[24] that can affect pigs in a production setting. Biannual random checks are performed for the research herd based on FELASA recommendations[25,26], in addition to samples from pigs that warrant diagnostics due to unexpected research results. Some of the pigs used for this project were seropositive for porcine reproductive and respiratory syndrome; however, none of the pigs showed any clinical symptoms corresponding to infection. All samples evaluated using polymerase chain reaction were negative, corroborating that the pigs were in a healthy condition.

PROTOCOL:

The pigs used to validate the protocol were part of studies approved by The Animal Experimental Council - a national governmental institution evaluating and approving all animal experiments in Denmark. The Council does the ethical review similar to an IACUC.

1. Arrival at the research facility

1.1. First, wash the pigs in a designated area to allow a thorough visual inspection. Use an exclusion list to exclude pigs with wounds, ear bites, abscesses, lameness etc., so only animals that appear clinically healthy are allowed into the facility.

1.2. After washing, sort the pigs into groups according to color marks. The color marks indicate which pigs have been pen mates at the supplier. This is important since pigs will fight to establish a hierarchy when they are introduced to new pen mates. Keeping them sorted in groups together with pigs they are already familiar with limits this fighting considerably.

1.3. Leave the pigs undisturbed (except when they are fed in the afternoon) for the rest of the arrival day as they can be quite stressed after transportation.

2. Part one: The transition phase (estimated time 4-6 days)

NOTE: The pigs stay in their home pen during the activities performed in the transition phase. We use half an apple for each treat-session. The progress of each individual pig is scored in the Transition Phase Progression Checklist (**Figure 1** and **Supplementary File 1**).

2.1. On the two consecutive days after arrival, introduce the treats.

2.1.1. Toss treats a few at a time (half apple total) into the pen twice daily to allow the pigs to search for the treats on their own and stand quietly just outside the pen. Apart from becoming familiar with treats, the goal is for the animal to positively associate the treat with the presence of a caretaker.

2.2. On day three and successive days introduce familiarizing the pig with “handling by a caretaker”. Enter the pen during feeding.

2.2.1. Sit down at arm’s length from the pig while it is eating (**Figure 3**). Calmly extend a hand towards the pig to touch its back. The touch should be more than two seconds to count as successful. If the pig backs away from the food, withdraw the hand slowly.

2.2.2. Wait until the animal commences eating and perform the action again. If the animal backs away from the food again, stop trying to touch it but remain inside the pen next to the trough (2-3 minutes in total for the session).

2.3. Move on in the handling procedure when the pig accepts being touched on the back while it is eating. Now move the hand around on the back in both direction of the head and the tail.

2.3.1. Touch the head and ears if the pig accepts it. If at any time, the pig backs away from an action stop the action but remain inside the pen for the remaining time (2-3 minutes in total for the session). The ear is important as it enables blood sampling from an intravenous catheter in the ear.

2.3.2. Rise slowly to be able to touch the tail region which is the second important area. Standing and moving about is more frightening to the pig, which is why this step is scored independently.

When the pig accepts having the area touched and the tail manipulated, a rectal temperature can be taken to support most research protocols.

2.4. To further strengthen the bond between caretaker and animal enter the pen during two sessions apart from the feeding times. Offer the pig treats (one slice at a time) during two sessions (2-3 minutes each), while sitting in a corner inside the pen. At first, give the treats from an outstretched arm, but decrease the distance gradually so the pig needs to come closer to get its reward.

2.5. The final stage of the transition phase is being able to touch the pig in the specific areas of the body and using only a few treats to do so. Enter the pen while a second caretaker stands outside the pen and offers a treat or two to the pig. Reward the pig for being calm and allowing touching as described and at the same time being capable of keeping all four hoofs on the ground (not jumping up on the fence of the pen).

3. Part two: Commencing clicker training

NOTE: Following the transition phase, the animals are ready to start the clicker training. It is recommended that the training is performed in a designated area as it helps the pig to recognize that a training session is about to begin. Several short sessions across the day are better than one longer session.

3.1. Take one pig at a time outside the home pen and into the designated training area. If the area used is novel to the pig, the first sessions should be without training and strictly exploratory with a few treats during about 5-7 minutes exploration per pig.

3.2. Introduce the clicker sitting or standing with the treats out of reach of the pig. Click when the pig shows interest, and immediately give a treat. Do this in quick succession while the pig maintains interest. If the pig loses interest, pause the click-and-treat until the pig makes contact again. This session should be no longer than 10 minutes.

3.3. The pig will understand the click quickly, but it is important to make the association between click and treat very strong. Repeat the step a few sessions in a row to make the association between click and treat strong. Test if the pig associates the click with a treat by clicking once and not following this with a treat and monitoring the behavior of the pig closely. If the pig looks around eagerly expecting the treat, the training is complete, if not perform a few additional sessions.

3.4. Once the click is known by the pig, introduce the target stick. A target stick is a stick with a marked area on one end. Hold the target end in the height of the snout. At some point, the pig will touch it out of curiosity. Click in the very second that the pig touches the target and give a treat.

3.5. Present the target again immediately and if touched click again and give a treat. Continue the target training until the pig understands that it will be rewarded for touching the target. Move the target gradually further away so the pig has to move further to get to the target.

3.6. When the pig follows the target when it is moved around add additional challenges by putting objects into the training area, so the pig has to concentrate harder on the target stick task to get its rewards.

REPRESENTATIVE RESULTS:

Applying the transition phase as described in the protocol's part one allows for a smooth introduction of farm pigs to the research facility. Having calm pigs to work with eases daily chores and enables minor procedures and sample taking to be performed without anesthesia or fixation. To follow the progress of pigs during the transition phase a checklist (Checklist 1) was developed to mark when individual pigs reach defined steps in their transition. The pigs are scheduled to enter research projects within two weeks of arrival and the total number of days was the measure chosen to show the success of the transition.

Based on Checklist 1, the transition phase of 20 pigs has been followed to verify that it is possible to transition pigs from farm pigs to research pigs within two weeks (**Figure 4**). All pigs completed the list within 12 days, showing that the timeframe set was sufficient.

Checklist 1 was modified based on the first experiences. A new stringent checklist (**Checklist 2**) was developed. To improve and standardize the transition further, the major revision was increasing the number of daily sessions and limiting the amount of time spent per session. Comparing the two checklists shows that pigs transitioned using Checklist 2 had similar progression during the first days of transition, whereas the later steps were reached quicker. A comparison between the use of Checklist 1 and Checklist 2 can be seen in Figure 5.

After the completion of the transition phase the animals will allow basic outcome measures like taking the temperature and inspection of surgical wounds. When a pig (female) has been transitioned to allow touching of the tail area a urine sample can be collected using an ostomy bag placed around the external genitals but under the anus (**Figure 6**).

Another example of a data outcome that can be collected is a blood sample. At our facility we often place a venous catheter in an ear vein during surgery. Following recovery, the catheter can be used to administer medicine to reduce the number of intramuscular injections, take multiple blood samples to support the project and evaluate the pig's return to normal physiology (**Figure 7**).

Moving on from the transition phase the clicker training will build on the transition phase to allow further benefits during handling and to obtain outcome data. Training the pigs to follow a target stick will allow the caretaker to move the pig e.g. onto a scale to monitor bodyweight (**Figure 8**) or into a transport crate (**Figure 9**) if the pigs need to be transported to surgery or an imaging facility. For one project, the pigs were trained to stand still while medicine was administered in

the eyes (Figure 10).

When starting the clicker training it becomes apparent that each pig is unique and they respond differently to the training. During training, this is acknowledged and training sessions are accommodated accordingly. Some pigs need two or three times as many training sessions to learn the same task, which can be problematic if certain tasks need to be learned at specific time points to support the research project.

FIGURE AND TABLE LEGENDS:

Table 1: Basic characteristics of the pigs used to evaluate the checklists.

Figure 1: The transition phase checklist. As illustrated, several of the activities take place on the same day but at different time intervals. Steps 3-5 takes place during feeding whereas steps 1-2 and 6-7 take place at defined time points during the day. Notice that each session must be performed during the time indicated on the top of the checklist but only takes approximately 2 minutes to carry out per pig. The shaded boxes indicate that the task is not to be performed. A full checklist can be found in supplementary file 1.

Figure 2: Some of the enrichment used at the department

Figure 3: Illustration of the position of the caretaker during steps 2.2 and 2.3 of the protocol.

Figure 4: Timeline of the transition phase using Checklist 1.

Figure 5: Progression of transition phase**.** A scatterplot showing the progression of the transition phase using Checklist 1 (◇) compared to Checklist 2 (O). Each plot corresponds to the observation for one pig. Mean and SD are indicated by the error bars. The y-axis is the number of days. On the x-axis the steps are referred as follows: 1; Apples from the hand. 2; Touch the pig during feeding. 3; Touch the hind and tail during feeding. 4; Touch the hind and tail while giving treats.

Figure 6: Urine sample collection via ostomy bag placed around outer genitals during feeding. The pig remains calm during the procedure and takes no notice of the ostomy bag.

Figure 7: Intravenous dosing during feeding after transition phase. The caretaker is able to sit in close proximity to the animal and focus on infusing the medicine correctly.

Figure 8: Stepping onto scale using target stick. The pig is eager to follow the target stick due to the positive reinforcement training.

Figure 9: Following target stick into transport crate. This procedure is useful if the pig needs to be transported to e.g. an imaging facility.

Figure 10: Pig trained to place its head in a holder and stand still so medicine can be given in the eyes without sedation. After the medicine is given, the pig rests the head on the holder and a

visual eye inspection can be performed.

DISCUSSION:

The implementation of a protocol that includes part one, a transition phase checklist and part two, a consecutive clicker training program is a confirmed success. Introduction and use of a detailed checklist have provided a way to refine the introduction of pigs to the research facility. The structured transition phase furthermore creates a better foundation to train the pigs prior to inclusion in research projects. It has been a positive surprise to notice that the caretakers' commitment to the work increases and that the positive emotions the pigs experience is reflected in their daily behavior.

The arrival of new pigs and the smooth transition to research pig has long been an area of focus but changes have been difficult to introduce. To create an overview of the work and the benefits it was decided to use a checklist to monitor the effectiveness of the prioritized steps. In Checklist 1, the steps were based on actions expected to have most impact on transition. The transition of 20 pigs were scored and evaluated using Checklist 1. Then the list was adjusted to eliminate the encountered problems. These mostly related to misunderstandings and inconsistent reporting. A critical step was the interpretation of each step and which actions the step involved. Hence, the description of each step was elaborated and the checklist was modified to be more intuitive. One step was removed to simplify the distinction between success and failure when touching the pig. To make it clear whether the step was performed or not a checkbox to indicate if the step was performed (✓) was added in addition to a box stating if the pig performed the action (+) or refused to perform the action (÷). To support the modifications additional time was allocated to train the use of Checklist 2. This discussion took place in groups with the aim to discuss each step and agree upon the success criteria. In relation to the practical execution, important issues were raised. For instance, when the pigs are used to the caretakers and treats, they can be very intrusive which can be intimidating and even dangerous to the caretakers. As a last modification, the number of sessions with each pig was increased while the time spent on each session was reduced.

To evaluate Checklist 2, 20 pigs were included. The data for step 4 is missing for three pigs, due to miscommunication between veterinarian and caretaker and underscores the need for communication. The time used daily is estimated to be the same, but the pigs complete the transition phase in fewer days. Having defined certain time slots during the day where the steps needed to be performed demanded a change in some daily routines. In combination with the total time needed to prepare and perform the steps this has been the biggest limitation in the implementation. The checklists contain 7 steps each, but due to the modification of the list the comparison in figure 3 is reduced to four comparable steps. Implementing a thorough structure during the transition phase creates awareness and makes the refinement initiative a priority in a busy research facility.

Implementing a structured checklist has enabled an evaluation of the variation between the individual pigs. It was interesting to notice that for some pigs, the number of days until they understood the treat was often higher compared to completion of the steps related to touching

by the caretaker. Another interesting observation was a rather sudden change in the willingness to be touched. One session, the pig would back away instantly, and at the next session only a few hours later the pig would allow for touching for long periods (more than a minute at a time).

To further prepare pigs for research projects, positive reinforcement training is started following the transition phase. A clicker training program is recommended. It allows for several caretakers in shifts to participate in the training of the animals. Using the clicker eases the shift between caretakers because the reward signal remains the same. When the clicker training commences it becomes evident that each pig is unique and responds to the training differently. Within a few sessions, however, all animals are eager to participate in the training sessions and fully enjoy the time spent with the caretaker. Clicker training the pigs enables specific tasks in relation to research projects to be carried out without using force. It also reduces the inter-animal variation seen in some projects, e.g. gait analyses. It can also be of great help in the daily chores, e.g. when the pig is moved within the research facility or is weighed. A critical step in the clicker training is the level of trust between pig and caretaker when the clicker is introduced. The pig needs to trust the caretaker as it needs to come close but remain focused on the task instead of the caretaker. Another critical step is the choice of treat. The animal must perceive it as a reward. Occasionally we come across a pig that does not find apples tasty and we then swap the apple with e.g. a piece of date or prune. It is important to consider the risk from working with large pigs, especially when they are excited in a training setting. They have a powerful bite, and they can jump up in an attempt to reach treats at unexpected time-points. To counteract these dangers, consider using the target stick to direct the attention away from the trainer, keep the treats clearly out of reach, work in short sessions, and do not run out of treats.

Working with the focused transition and training of pigs has brought with it an increased enthusiasm from the caretaker staff. Education in the field of positive reinforcement training has been a priority and given rise to the fact that caretakers experience a stronger commitment to the research projects. Involvement of the caretakers in the hands-on planning of new projects in dialogue with the researcher and the veterinary staff enables the caretakers to give qualified suggestions on how training could support the research results *and* the animal welfare. The collection of more data from each pig without causing harm is one of the cornerstones of the 3Rs. Weekly meetings focused on the transition and training have increased the focus on each individual pig and enhanced the department's Culture of Care. Seeing the pigs when they are about to start a training session is rewarding in itself, and the behavior more than suggests that the pigs enjoy the sessions. When planning the training of pigs for specific projects, bear in mind that the pigs will enter a state of excitement when they get familiar with the training routines. Ideas to counteract this include; If possible, do not train where samples are to be collected. So if you plan to do blood samples in the home pen, try to do the training somewhere else. The pigs will know their daily routine, so plan to take samples at time points where training is not usually performed if possible. When training has to be where the samples are to be collected, let the pig explore the area for a set number of minutes each time, before beginning the training or data collection protocol.

Placing a structure on our transition phase has been an overall positive initiative. A reduction in

the stress of the pigs shortly after arrival and a quick transition to having pigs that enjoy the company of the caretakers rather than hurtling about the pens is evident. We have experienced a turnaround in the staff from a point of view where they only saw extra work, to the point where they see the benefits of transitioning the pigs using the checklist. The protocol has an immense application potential and can be applied as an important tool in almost every set up involving research pigs. We now use it to transition both farm pigs and minipigs and aim to implement the clicker training even further in projects related to e.g. cardiovascular science, neuroscience and ophthalmology. The general attitude and Culture of Care in the department has been raised and there is a wish and eagerness to propel these new experiences further to merge research and animal welfare continuously and apply the 3Rs.

ACKNOWLEDGMENTS:

Beatrice Tscherning Olesen, Karoline Jakobsen, Tine Bjørnholt Gustafsen, Helena Céline Kjær Byriel and Kira Sonnichsen Graahede are acknowledged for their great efforts, feedback and assistance in designing the checklist and using it in their daily work. Medical illustrator Ken Kragsfeldt is acknowledged for his professional help illustrating figure 3.

DISCLOSURES:

The authors have no disclosures.

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Figure 1

Click here to access/download;Figure;Figure 1_Transition phase progression checklist.pdf

Animal ID:

Arrival date: 23. juli 2020

Transition phase progression checklist

Caretaker initials:																		
Date:	23-07-2020		24-07-2020		24-07-2020		25-07-2020		25-07-2020		26-07-2020		26-07-2020		26-07-2020		26-07-2020	
Time:	Whole day		09:00-10:00		12:00-13:00		09:00-10:00		12:00-13:00		07:00-07:30		09:00-10:00		12:00-13:00		14:00-14:30	
Activity - Elaborated under Explanation	(✓)	(+ / -)	(✓)	(+ / -)	(✓)	(+ / -)	(✓)	(+ / -)	(✓)	(+ / -)	(✓)	(+ / -)	(✓)	(+ / -)	(✓)	(+ / -)	(✓)	(+ / -)
Apples on the floor (2 min)																		
Apples from hand (2 min)																		
Touch during feeding **																		
Touch head, ears and neck during feeding**																		
Touch hind and tail during feeding**																		
Touch head, ears and neck using treats**																		
Touch hind and tail using treats**																		

Figure 2

Click here to access/download;Figure;Figure 2_Enrichment.pdf

Figure 3

Click here to access/download;Figure;Figure 3_Position of the caretaker.pdf

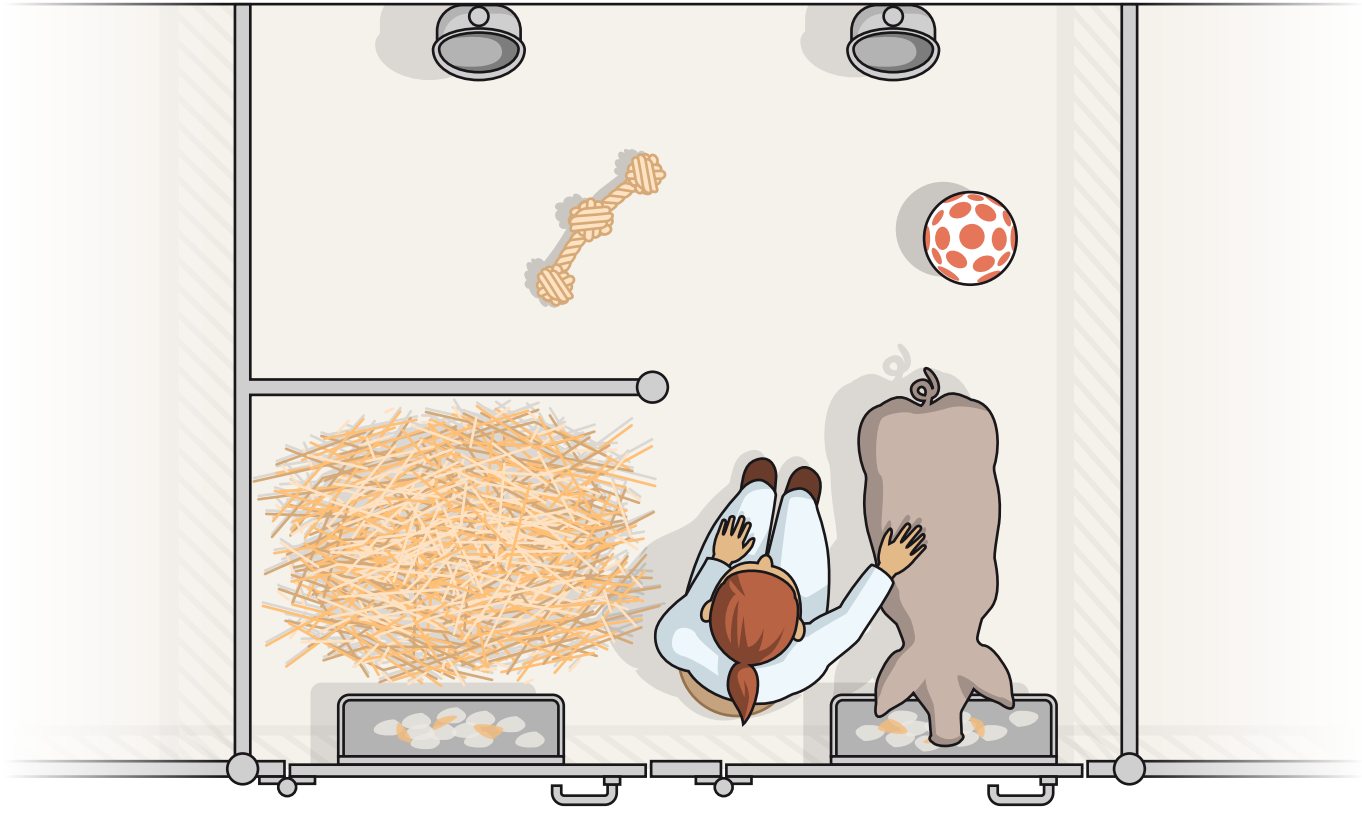

Figure 4

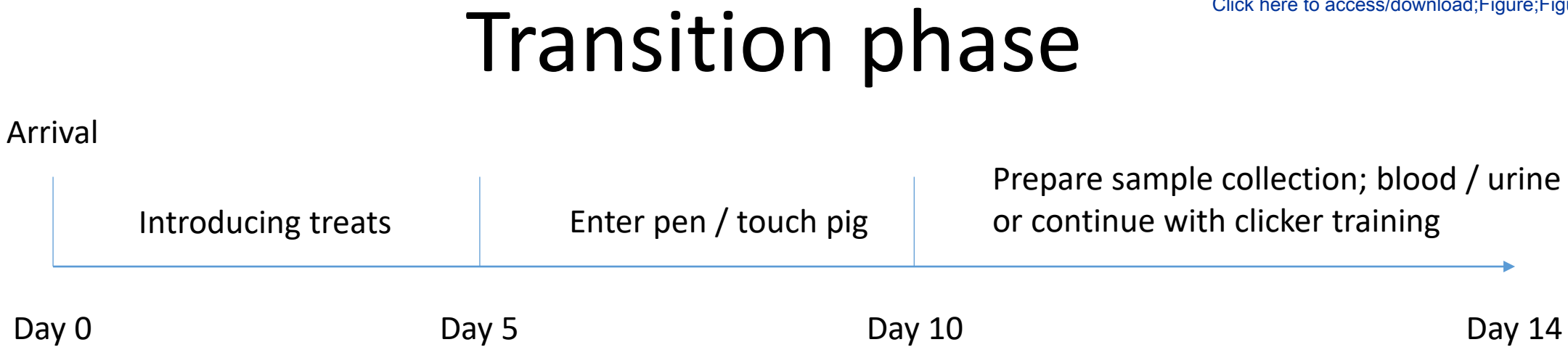

Figure 5

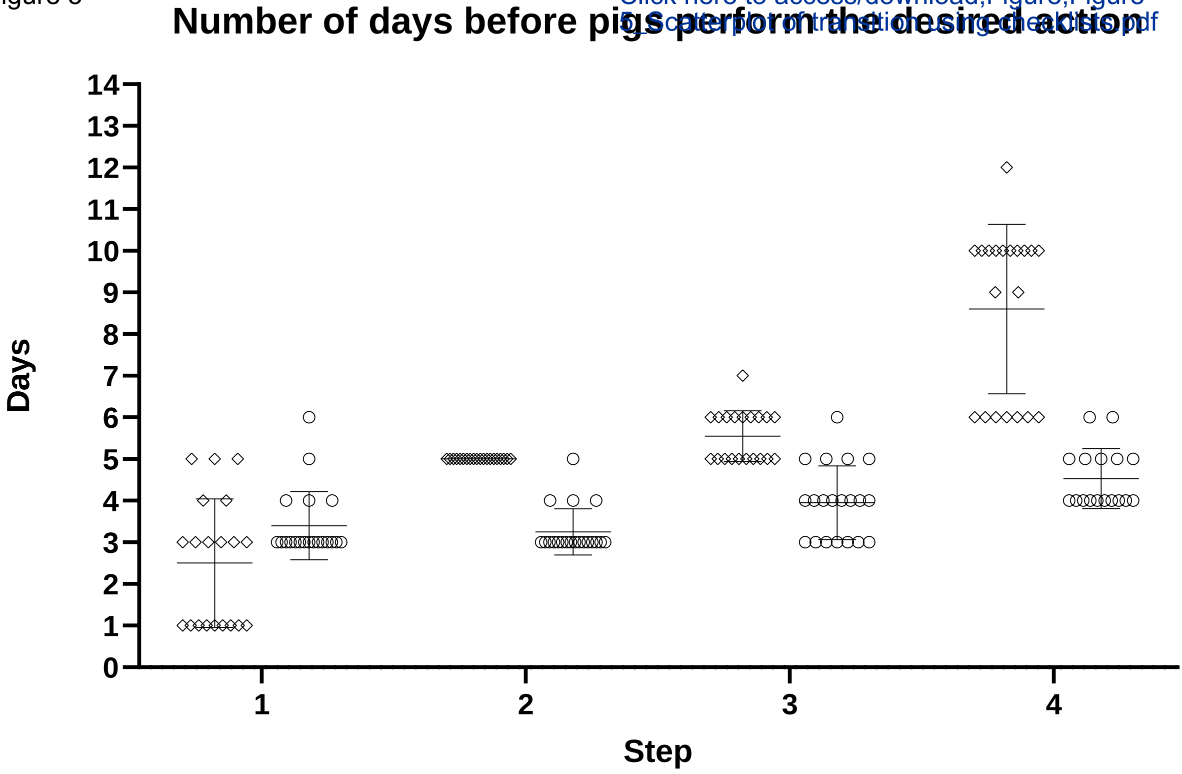

Figure 6

Click here to access/download;Figure;Figure 6_Urine sample via ostomy bag.pdf

Figure 7

Click here to access/download Figure Figure 7_Pig IV dosing.png

Figure 8

Click here to access/download;Figure;Figure 8_Pig walking onto scale.pdf

Figure 9

Figure 10

Click here to access/download;Figure;Figure 10_Pig trained to have eye drops.pdf

Table 1

Animal data	Checklist 1 (n=20)
Species	Domestic pig (Sus scrofa domesticus)
Breed	(Danish Landrace x Yorkshire) x Duroc
Sex	Female
Weight	35-80 kg (mean 47,5 kg)
Age	10-22 weeks

Updated checklist (n=20)
Domestic pig (Sus scrofa domesticus)
(Danish Landrace x Yorkshire) x Duroc
Female
40-80 kg (mean 62,5 kg)
10-22 weeks

Dear editor, reviewer #1, reviewer #2, reviewer #3 and veterinary reviewer,

We appreciate the large effort put into reviewing our paper and film, and thank for all the comments. The paper has been revised accordingly.

Please find our point-by-point replies below.

Response to editorial and production comments:

Comment 1.

Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

Response 1:

Thank you for drawing attention to this. We agree that correct spelling and grammar is important and the manuscript is now proofread by an English linguist. We trust this has eliminated spelling and grammar issues.

Comment 2.

Please include an ethics statement before your numbered protocol steps, indicating that the protocol follows the animal care guidelines of your institution.

Response 2:

Thank you for this comment. The pigs were housed according to the Institution's Animal Welfare Policy, which refers to national legislation on animal experimentation, housing and husbandry. The statement has been added, see lines 133-134.

Comment 3.

Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., "Do this," "Ensure that," etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as "could be," "should be," and "would be" throughout the Protocol.

Response 3:

Thank you for drawing attention to the protocol format. We recognize that the imperative tense is important. We have revised the protocol and changed the wording to imperative and included a short passage in the discussion that briefly touches upon the subject of the inter-animal variation when it comes to animal temper and trust.

Comment 4.

The Protocol should contain only action items that direct the reader to do something. Please move the discussion about the protocol to the Discussion.

Response 4:

Thank you once again for drawing our attention to this. We have removed the discussion from the protocol section as requested.

Changes to be made by the Author(s) regarding the video:

Video changes 5:

Title
Please present an ethics statement card before the protocol section of the video.
00:01 - 00:14 ""The titles formatting looks a bit off. Some words are bold while others are thinner. Please format the text so they are all the same weight.
At the same time, please capitalize the first letter of every word so it's more of a title and less of a sentence.
Lastly, to shorten the runtime of these titles, please place the authors and affilliates on the same screen as the title by placing credit under the title."""

Response 5:

Thank you for these comments. We agree and have added an ethics statement as requested. The title's formatting has been changed and the credits added to the first slide. Title: "Transition of Farm Pigs to Research Pigs Using a Designated Checklist Followed by Initiation of Clicker Training – a Refinement Initiative"

Video changes 6.

Pacing
00:15 - 00:15 The title shouldn't be 15 seconds long. Let's limit the time to 5 seconds.
05:16 - 05:19, 07:34 - 07:48, 07:52 - 08:01 These shots goes on for too long after the narration ends. Please cut to the next shot and following narration sooner:

08:33 - 08:49 We normally don't add credits to the end. Also, this part is a bit too long. Let's try to combine the last two slides into one (Video/Editor and Narrator). Also the credit of ""Speak"" is worded weirdly and should say ""Narrator"" or ""Narrated by"".

Response 6:

Thank you for directing attention to this. We appreciate the comments and have revised in the following manner:

00:15 - 00:15

We have shortened the view time as requested.

05:16 - 05:19

We have removed those 3 seconds.

07:34 - 07:48

We have reduced in the scene but left enough time to appreciate how calm the pig is while the gate to the crate is closed behind it.

07:52 - 08:01

We have reduced the time of this scene as requested, by limiting the time showing the pig walking towards the floor scale.

08:33 - 08:49

We have deleted the acknowledgements to shorten the time used on text slides and combined the last two slides, and moved them to the beginning of the video.

Video changes 7.

Composition
00:16 - 00:29 There's an obvious blur effect on this shot for some reason and un blurs after 15 seconds into the narration. Please remove this blur effect.
00:58 - 01:00 There's a fake blur effect being applied here. Please remove it since it serves no purpose.
02:33 - 02:33 The camera shakes here. Let's try to cut this part out by changing to another shot before the shake.
05:24 - 05:24 I'm not sure what's going on here, but it looks like the person on camera is yelling at the person off camera and it looks awkward. If there's another shot where the person on camera isn't yelling off camera, please replace this shot with that.

05:46 - 05:46 There's a black frame that pops up here. Also the camera does a quick zoom out after this black frame. Please remove the zoom out as well."

Response 7:

Thank you for these nice observations regarding the composition of the video

00:16 - 00:29

This blur was to force the attention unto the narration, we have removed it as requested. We have kept a slight blur on our supplier, as he is not part of our organization and due to General Data Protection Regulation (GDPR) we do not wish his face shown. It is our hope that such a solution is acceptable.

00:58 - 01:00

We removed the blur effect.

02:33 - 02:33

We cut out the shake.

05:24 - 05:24

We changed to another shot showing the area in question.

05:46 - 05:46

We removed the black frame and cut the scene before the zoom out.

Video comments by the veterinary reviewer:

Video changes 8.

0:42 As the pigs arrive, one of the caretakers is shown to kick towards a pig as it walks by. Although contact was not made, so there was no welfare issue, it looks as if the caretaker was attempting to kick the animal for no reason.

Response 8:

Thank you for directing our attention to this scene. We did not view the scene and the boot in the described manner, so it is nice to have the feedback. Just briefly, we only move pigs over shorter distances and the boots/feet of the caretakers serve as a visual blockade to make sure the pigs walk the intended way. We avoid using sorting panel or paddle stick since we find those to induce

more stress and carry no obvious benefits due to the short distance. However, we acknowledge your point of view and have removed the scene accordingly to avoid confusion.

Video changes 9.

02:38, 03:18, 04:29

At these sections of the video, the narrator is discussing procedures related to acclimation performed prior to starting the study. However, the pig shown already has a catheter in the ear vein, indicating that it already completed acclimation and started on study.

Response 9:

This is a good observation, thank you. To fulfill our commitment to reduce the number of animals used for experiments we do not use pigs only to work with these refinement initiatives. All the pigs used to validate the current protocol were destined for specific research projects. Due to the logistics of filming different time points we have tried to best illustrate the steps of the protocols with the pigs we had available. For the time points mentioned, the pigs showing the desired behavior had a catheter placed a few days previously as part of their destined research protocol. We find it important to illustrate the step in the protocol in a favorable way and believe it outweighs the fact that the animal has a catheter.

Reviewers' comments:
Reviewer #1:

Minor concern 10.
Title and lines 2, 80, 84, 133, 253: replace the word "conventional" [pigs] with "farm" [pigs], as it is used on line 261. The term "conventional" is normally used to describe the health status, therefore may create confusion.

Response 10:

Thank you for this comment. The wording was too directly translated from Danish, we agree with the proposed wording and have changed it accordingly throughout the manuscript. E.g., lines 1-2: “Transition of farm pigs to research pigs using a designated checklist followed by initiation of clicker training – a refinement initiative “

Minor concern 11.

Line 82: "both breeds" to be replaced with "both farm pigs and miniature pigs" (these 2 are not "breeds", there are several breeds of pigs and miniature pigs).

Response 11:

Thank you for this comment. The word breed is as stated incorrect. We have changed the wording see line 83-84. “Both farm pigs and miniature pigs can indeed be trained”

Minor concern 12.

Lines 102-106: I recommend to consult and reference the following publications:

A: Smith AC and Swindle M, Preparation of Swine for the Laboratory: https://doi.org/10.1093/ilar.47.4.358

B: McCrackin MA and Swindle M, Chapter 1. Biology, Handling, Husbandry, and Anatomy in Swindle, M. (Ed.), Smith, A. (Ed.). (2016). Swine in the Laboratory. Boca Raton: CRC Press, https://doi.org/10.1201/b19430

C: Sørensen DB et al, Positive Reinforcement Training in Large Experimental Animals, http://www.altex.ch/resources/477479_So776rensen21.pdf

D: Kaiser AR et al, Independent Study 490A: Positive Reinforcement Training Piglets to Stand in a Container and Follow a Human: https://lib.dr.iastate.edu/ans_air/vol660/iss1/79/

E: Herskin et al, Chapter 13. The Pig, in Sørensen DB (Ed.) et al., Animal-centric Care and Management: Enhancing Refinement in Biomedical Research, Boca Raton, CRC Press 2020, https://doi.org/10.1201/9780429059544

Response 12:

Thank you for suggesting these publications. We have read them and agree that they are relevant in our manuscript. We have added A+B in line 95 with regard to their focus on animal welfare and husbandry. We added C+D in line 107 as they are more specifically discussing positive reinforcement training. We have added E in line 121 as it underlines the emotional part of animal wellbeing where we see training as an obvious way of adding joy to the husbandry of pigs.

Minor concern 13:

Line 123: "department's culture of care". I would recommend to consult the following resources and add them as references:

F: Bertelsen T and Hawkins P, Chapter 2. A Culture of Care, in Sørensen DB (Ed.) et al., Animal-centric Care and Management: Enhancing Refinement in Biomedical Research, Boca Raton, CRC Press 2020, https://doi.org/10.1201/9780429059544

G: Weichbrod H. et al, Section II Developing a Collaborative Culture of Caring, in Management of Animal Care and Use Programs in Research, Education, and Testing, 2nd edition, CRC Press/Taylor & Francis; 2018: https://pubmed.ncbi.nlm.nih.gov/29787045/

Response 13:

Ad F+G

Thank you for directing our attention to these excellent references. We have struggled to find a reference for the concept of a culture of care. We have attended several conferences and seminars during the last 3-4 years where the concept has been touched upon, but we have lacked a good written introduction to the concept and now you have supplied us with two. We have added them as proposed in line 127.

Minor concern 14:

Line 140: please describe which toys are used.

Response 14:

Thank you for pointing this lack of information out. We have added a description to line 149: “toys e.g., rope, balls, buckets, chew sticks made from plastic (Figure 1). We also added a picture, as animal toys are hard to describe in words.

Minor concern 15:
Line 142: "they are screened for seven pathogens that can affect pigs in a production setting": the 7 pathogens should be listed. Is a clinical examination performed, in collaboration with the farm's veterinarian? I encourage the authors to consult and add as reference: Berset CM et al, Federation of European Laboratory Animal Science Associations recommendations of best practices for the health management of ruminants and pigs used for scientific and educational purposes: https://doi.org/10.1177/0023677220944461

Response 15:

This a relevant comment, thank you. The farm pigs are screened based on the national SPF system at the supplier’s farm (a detailed explanation is added as a reference). At the research farm a dedicated health monitoring program based on the previous FELASA recommendation and the new one linked her is used. We have made this clearer by implementing the following:

lines 152-156: “The animals come from a herd included in the national health-monitoring program for farm pigs, which means they are screened for seven pathogens (24) that can affect pigs in a production setting. Additional biannual random health monitoring is performed at the research facility. These are based on FELASA recommendations(25, 26)”

Reviewer #2:
Minor concerns 16:

I have concerns regarding the reproducibility of this protocol, though, as detailed information on how often per session and for how long to execute the described actions is partly in the text and partly in the checklist, and partly lacking. E.g., in 2.1, is the caretaker supposed to throw the treats in the pen all at once or small amounts repeatedly; in 2.2 how often should the caretaker try to touch the animal? To ensure a broad application of this helpful protocol a more detailed description is necessary.

Response 16:

Thank you for pointing out that the steps lack details to ensure reproducibility. The protocol has been revised to give additional information on the specific actions. Lines 193-208:

2.1. “On the two consecutive days after arrival, introduce the treats. Toss treats - a few at a time (half apple total) - into the pen twice daily to allow the pigs to search for the treats on their own and stand quietly just outside the pen. Apart from becoming familiar with treats, the goal is for the animal to positively associate the treat with the presence of a caretaker.
2.2. On day three and successive days introduce familiarizing the pig with “handling by a caretaker”. Enter the pen during feeding.
Sit down at arm’s length from the pig while it is eating (Figure 2). Calmly extend a hand towards the pig to touch its back. The touch should be more than two seconds to count as successful. If the pig backs away from the food, withdraw the hand slowly. Wait until the animal commences eating and perform the action again. If the animal backs away from the food again, stop trying to touch it but remain inside the pen next to the trough (2-3 minutes in total for the session).”

In addition, we have added a section to the discussion concerning the steps, and progression of the protocol. Lines 399-404: “Implementing a structured checklist has also enabled an evaluation of the variation between the individual pigs. It was interesting to notice that the number of days until the pigs understood the treat was often higher than the completion of the steps related to touching by the caretaker. Another interesting observation was a rather abrupt change in the willingness to be touched. One session the pig would back away instantly, and then the next session only a few hours later the pig will allow for touching for long periods (more than a minute at a time).”

Minor concern 17:

Line 82 ,both breeds' referring to 'conventional slaughter pigs' (l. 80) and 'miniature pigs'(l. 81) as breeds is taxonomically incorrect. Instead, there are various pig breeds within both categories, so please do not use the term 'breed' unless you want to refer to one of those.

Response 17:

Thank you for bringing this to our attention. The word breed is as stated incorrect. We have changed the wording to line 83-84. "Both farm pigs and miniature pigs can indeed be trained"

Minor concern 18:

A: Line 84ff 'Conventional pigs […] are housed in high density groups of 15-25 pigs where survival partially depends on their ability to defend themselves in the hierarchy of the herd.' First of all, group sizes might vary a lot from the range given here, from both smaller, single-digit numbers to large dynamic herds of breeding sows with 100 or more individuals.

B: Secondly, the picture the authors draw here of conventional pig farming is way too drastic. To my knowledge (from working with farmed pigs for 15 years) conventional hardly ever die from the consequences of intra-group competition, be it due to severe injuries from agonistic interactions or restricted access to food (growing and fattening pigs are fed ad libitum, and breeding sows are fed individually, either when housed in crates or with feeding stations in group-housing).

Response 18:

Ad A: Thank you for pointing this out. We have specified that in this context we describe finisher pigs. Lines 87-88 "Finisher pigs for meat production in Denmark are typically housed in high density groups of 15-25 pigs where they have to compete to earn a position in the herd's hierarchy."

Ad B: We agree survival in the direct meaning is the wrong word to use, as we agree that death is not usually the outcome of competing to establish a hierarchy. We have changed the wording as can be seen above. However, we still argue that farm pigs compete by fighting and this is seen more when they are confined to relatively small spaces compared to wild breeds in free range or miniature pigs as they are more docile.

We also use more specific terms regarding housing and state that farm pigs most often used for research (at our facility) are finisher pigs. We elaborate the meaning of a Danish setting.

Minor concern 19.

A: Line93f 'The welfare of the animals is important to ensure a low inter animal variation.' There are two aspects I want to address here. First, in how far does (good) welfare lead to low inter-individual variation?

B: And second, is low inter-individual variation what we should aim for? I might be misinterpreting this sentence, but to me it sounds like you advocate for low inter-individual variation to ensure good quality research outcomes. This viewpoint seems a bit outdated, as it has been argued repeatedly over the last two decades at least that less standardized, more heerogenous samples will produce more valid research results (e.g., see Richter SH, Garner JP, Würbel H (2009) Environmental standardization: cure or cause of poor reproducibility in animal experiments? Nat Methods 6: 257-261; Würbel H (2000) Behaviour and the standardization fallacy. Nat Genet 26: 263.; Lewejohann L, Reinhard C, Schrewe A, Brandewiede J, Haemisch A, et al. (2006) Environmental bias? Effects of housing conditions, laboratory environment and experimenter on behavioral tests. Genes Brain Behav 5: 64-72.)

Response 19:

Thank you for addressing this very interesting topic. We have changed the sentence to be clearer, lines 96-97: “The welfare of the animals is important to ensure a low inter-animal variation in regard to stress, anxiety and fear.”

Ad A: In the sense that poor welfare can give rise to stress related behavior, anxiety and fear. Each animal will have a different tolerance to these psychological challenges and so in a herd/group they will introduce a marked variation. This variation we wish to minimize. In the same way we aim to have a high health status in the animals to reduce the risk of variation from infectious diseases. We have included these references to clarify the statement.
See: https://www.sciencedirect.com/science/article/pii/S0739724002001467 ;
https://iv.iiarjournals.org/content/invivo/24/5/641.full.pdf

Ad B: The papers listed focus on rodents housed in cages that are very similar in terms of size and enrichment. As globally there are a limited number of companies producing housing systems for rodents, and they all make cages that only just comply with the housing legislation rodent housing is quite similar. So, the cages are close to identical, and if not equipped with enrichment they will create a “negative” low inter-animal variation that in reality resembles a depressed phenotype as the rodent lose some of their exploratory behaviors used in an array of behavioral test.

We agree that over-enforcing a “standardized” housing typically by removing enrichment is not the way forward. As described under the housing of our pigs we have a plan to stimulate the pigs

with as many different toys, smells and enrichments as possible. In addition, facilities for pigs are often build with local/national recommendations. Our own farm has five different types of pens/stables/sections due to varying construction years, so also in that aspect the housing conditions are varying and hard to compare to typical rodent housing. We argue that negative variation should be minimized, but “positive” variation created by simulating local environment should be encouraged.

Minor concern 20.

Line 348f 'when the pigs are used to the caretakers and treats, they can be very intrusive which can be intimidating to the caretakers.' This reflects my own experience, with being 'intrusive' also including biting, so it is not just intimidating but poses a safety threat for the caretakers. Which action do you recommend to your caretakers?

Response 20:

Thank you for putting our attention to this important issue. We have experienced biting as well. Especially if the pigs in question are large this can pose a danger. In our facility the size limit is typically around 120 kg. We have added information in the discussion addressing this danger, and recommendations as to avoid being caught in a dangerous situation. See lines 421-425: “It is important to consider the risk from working with large pigs, especially when they are excited in a training setting. They have a powerful bite, and they can jump up in an attempt to reach treats at unexpected time-point. To counteract these dangers, consider how to use the target stick to direct the attention away from the trainer, keep the treats clearly out of reach, work in short sessions and don’t run out of treats.”

Minor concern 21.
Just one thing came to mind: I think it would be good to address (in the article or the video) how to avoid pigs always expecting rewards. With some experiments, it can be problematic if subjects expect to be constantly rewarded by any person coming into their pen or interacting with them otherwise. They can either get overly excited when seeing humans (resulting in a physiological stress response), or get frustrated if there is no reward. In my experience, it is best if pigs do consider experimenters to be neutral rather than negative (handling without prior habituation training) or positive (rewards on each encounter of a human caretaker or experimenter). I would be happy to hear the author’s point of view.

Response 21:

This is an interesting observation and one that we do encounter from time to time. Briefly, what we generally try in this regard is train in a designated area and not in the home pen. In the home pen we often take samples and that can be affected by excitement. For some behavioral studies training has to be performed in a specific room due to equipment or similar, and when that is the

case and we can see no other way of making the pig perform a task we train and reward rather than force/handle the pig to make it perform the task.

Based on this comment we have added a few lines regarding this issue in the discussion, lines 437-444: “When planning the training of pigs for specific projects, bear in mind that the pigs will enter a state of excitement when they get familiar with the training routines. Ideas to counteract this include; If possible, do not train where samples are to be collected. So if you plan to do blood samples in the home pen, try to do the training somewhere else. The pigs will know their daily routine, so plan to take samples at time points where training is not usually performed if possible. When training has to be where the samples are to be collected, let the pig explore the area for a set number of minutes each time, before beginning the training or data collection protocol.”

Reviewer #3:

Minor Concern 22.
You can decrease some of the introduction to focus strictly on refinement without going into as much background on the rodent or farm pig husbandry. It's not imperative, but is more than is needed to still discuss why training is important for refinement.

Response 22:

Thank you for this comment. We believe that the background information is important to increase the reach of the manuscript. Much of the information is quite common knowledge for researchers, caretakers and veterinarians in established research facilities in well developed countries. We hope that this manuscript can encourage less experienced researchers and staff to implement the protocol as well as encourage institutions in countries that are still in the very beginning of implementing the 3Rs. Thus we suggest to keep the introduction with the current broad perspective.

Minor concern 23.

Line 86 - "survival" isn't dependent on hierarchy per say but their place in the hierarchy does determine stress reactivity

Response 23:

Thank you for this comment. We have changed the sentence to make it more precise. Lines 87-88 “Finisher pigs for meat production in Denmark are typically housed in high density groups of 15-25 pigs, they have to compete to earn a position in the herd’s hierarchy.”

Minor concern 24.

Any time you refer to the pigs as "slaughter pigs" - change to domestic pigs, or even farm pigs if you prefer.

Response 24:

Thank you for bringing this issue to our attention. Slaughter pig is a too direct translation from the Danish word for this kind of pig. We have changed both slaughter pigs and conventional pigs to farm pigs throughout the text.

Minor concern 25.

I would change your "Note:" header to something like "General comments" or "Overview" since it reads like a summary before going into steps of how to train.

Response 25:

Thank you for this suggestion. We agree that the word note is not covering as a header for the content prior to each step in the protocol. We have changed “note” to “General considerations” in lines 179 and 234.

Minor concern 26.

Transition Phase section - can you include a sentence about it being important to or OK to start during the acclimation period. In the US, USDA requires a minimum of 5d acclimation for pigs and some people may think that to mean you can't start training during that time, when I believe socializing them during this period is extremely important.

Response 26:

Thank you for this comment. We have added a sentence in the background, underscoring that no harmful actions are performed as part of the protocol, emphasizing that it can indeed be initiated just after arrival. See lines: 142-144: “As the protocol is composed of non-harmful and positive actions, it can be initiated just after arrival, without prior acclimatization period.”

Minor concern 27.

In the steps (e.g., 2.2/2.3)

A: Is it important to repeat these steps daily until the pig allows touch?

B: Is there a certain amount of time frame the pig should allow you to touch to count as a successful step before moving forward? I think you address that pigs can take longer in each of these steps later on but it should briefly be mentioned during the methods.

Response 27:

Thank you for directing our attention to this issue.
Ad A, Yes, these steps should be performed twice daily as stated in the protocol. They are performed while the pigs get their feed, which in our case is two times daily, in the morning and in the afternoon.
B: We have tried to add more information in the text with a time limit to the step itself (Lines 203-208: "Calmly extend a hand towards the pig to touch its back. The touch should be more than two seconds to count as successful. If the pig backs away from the food, withdraw the hand slowly. Wait until the animal commences eating and perform the action again. If the animal backs away from the food again, stop trying to touch it but remain inside the pen next to the trough (2-3 minutes in total for the session)." In general, it is often very obvious. One session the pig will back away from the touch, the next it will allow touching even for long periods, so at least in our experience it is really a yes or no answer. If the pig backs away two times in a row the caretaker stops trying to touch, but remain inside the pen for the rest of the 2–3-minute session. We have added a brief discussion of pigs' behavior during transition regarding this issue as well. See lines: 399-404.

Veterinary review of the manuscript:

Minor concern 28.

The pigs were individually housed without providing a scientific justification as to why individual housing was needed. Swine are social animals, and Social animals should be housed in stable pairs

or groups of compatible individuals unless they must be housed alone for experimental reasons or because of social incompatibility.

Response 28:

Thank you for bringing this to our attention. We fully agree that pigs should be housed together whenever possible. A statement addressing this is added in the text. Line 147-149: "There is a feeding trough for each pig and at least one section of the separation to the neighboring pens allow snout contact. When pigs are planned to enter surgical interventions or have implants of any kind, they are housed alone to avoid pen mates licking and biting in wounds and pulling implants out."

Minor concern 29.

In the text, the authors state "Historically they [rodents] have been treated quite poorly"…

While rodents are handled differently than swine, primates, etc., I disagree with the statement that researchers treat their rodents poorly. I believe that most research rodents receive good husbandry and veterinary care. I believe this statement should be revised.

Response 29:

Thank you for an interesting point of view. However, we do argue that previously e.g., analgesia was not routine treatments in many research institutions, and even when used it was often insufficient to the interventions performed. See e.g.:
https://www.altex.org/index.php/altex/article/view/780

Many reviews have corroborated these findings of in-adequate analgesia protocols. We believe that reasons are not due to deliberate ill-intend, but rather due to lack of knowledge in regard to pain management in animals...
In regard to rodents, we also notice that full-grown rats may be offered enrichment but are not allocated enough space to perform normal behavior. Studies have described how rats used in research are different from their wild relatives performing a reduced number of the normal behaviors seen in wild rats. In our opinion there is still much to improve.

We have however changed the sentence at bit to soften the wording and accommodate your point of view. See line 71-72: "They have not received equivalent public attention and care. Historically they have been treated less well"

Minor concern 30.

Acronyms should be spelled out and explained the 1st time they are used, e.g., "porcine reproductive and respiratory syndrome (PRRS)

Response 30:

Thank you for bringing these acronyms to our attention. We have removed them as acronyms as they are not used later in the text. Lines 156-159: ".. seropositive for porcine reproductive and respiratory syndrome, however, no pigs showed any clinical symptoms corresponding to infection. All samples evaluated using polymerase chain reaction were.."

Minor concern 31.

I believe the word "central" should be dropped. Central venous catheters normally refer to those threaded into large veins such as the central vena cava. The catheter in the ear vein would simply be a venous catheter.

Response 31:

Thank you for this comment, we agree and indeed the catheter used is a central venous catheter inserted from the base of the ear. We usually use 20-25 cm central catheters as in our experience the pigs clot smaller venous catheters way too often for them to be useful for more than a day or so. Using the central venous catheters, we can routinely draw blood daily for up to about two weeks. However, for the sake of the example the type of catheter is irrelevant, we have removed the word "central" in the sentence. See lines 303-304: "Another example of a data outcome that can be collected is a blood sample. At our facility we often place a venous catheter in an ear vein during surgery."

Supplemental File (Transition Checklist))

Click here to access/download;Supplemental File (Figures, Permissions, etc.);Transition phase progression checklist.xlsx

Animal ID:	Transition phase progression checklist																											
Arrival date:																												
Caretaker initials:																												
Date:	7/23/2020		7/24/2020		7/24/2020		7/25/2020		7/25/2020		7/26/2020		7/26/2020		7/26/2020		7/26/2020		7/27/2020		7/27/2020		7/27/2020		7/27/2020			
Time:	Whole day		09:00-10:00		12:00-13:00		09:00-10:00		12:00-13:00		07:00-07:30		09:00-10:00		12:00-13:00		14:00-14:30		07:00-07:30		09:00-10:00		12:00-13:00		14:00-14:30			
Activity - Elaborated under Explanation	(✓)	(+ / -)	(✓)	(+ / -)	(✓)	(+ / -)	(✓)	(+ / -)	(✓)	(+ / -)	(✓)	(+ / -)	(✓)	(+ / -)	(✓)	(+ / -)	(✓)	(+ / -)	(✓)	(+ / -)	(✓)	(+ / -)	(✓)	(+ / -)	(✓)	(+ / -)		
Apples on the floor (2 min)																												
Apples from hand (2 min)																												
Touch during feeding **																												
Touch head, ears and neck during feeding**																												
Touch hind and tail during feeding**																												
Touch head, ears and neck using treats**																												
Touch hind and tail using treats**																												

Caretaker initials:																												
Date:	7/28/2020		7/28/2020		7/28/2020		7/28/2020		7/29/2020		7/29/2020		7/29/2020		7/29/2020		7/30/2020		7/30/2020		7/30/2020		7/30/2020					
Time:	07:00-07:30		09:00-10:00		12:00-13:00		14:00-14:30		07:00-07:30		09:00-10:00		12:00-13:00		14:00-14:30		07:00-07:30		09:00-10:00		12:00-13:00		14:00-14:30					
Activity - Elaborated under Explanation	(✓)	(+ / -)	(✓)	(+ / -)	(✓)	(+ / -)	(✓)	(+ / -)	(✓)	(+ / -)	(✓)	(+ / -)	(✓)	(+ / -)	(✓)	(+ / -)	(✓)	(+ / -)	(✓)	(+ / -)	(✓)	(+ / -)	(✓)	(+ / -)				
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Touch hind and tail using treats**																												

Animal ID:	Transition phase progression checklist																											
Arrival date: 23. juli 2020																												
Caretaker initials:																												
Date:	7/31/2020		7/31/2020		7/31/2020		7/31/2020		8/1/2020		8/1/2020		8/1/2020		8/1/2020		8/2/2020		8/2/2020		8/2/2020		8/2/2020					
Time:	07:00-07:30		09:00-10:00		12:00-13:00		14:00-14:30		07:00-07:30		09:00-10:00		12:00-13:00		14:00-14:30		07:00-07:30		09:00-10:00		12:00-13:00		14:00-14:30					
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Caretaker initials:																												
Date:	8/3/2020		8/3/2020		8/3/2020		8/3/2020		8/4/2020		8/4/2020		8/4/2020		8/4/2020		8/5/2020		8/5/2020		8/5/2020		8/5/2020					
Time:	07:00-07:30		09:00-10:00		12:00-13:00		14:00-14:30		07:00-07:30		09:00-10:00		12:00-13:00		14:00-14:30		07:00-07:30		09:00-10:00		12:00-13:00		14:00-14:30					
Activity - Elaborated under Explanation	(✓)	(+ / -)	(✓)	(+ / -)	(✓)	(+ / -)	(✓)	(+ / -)	(✓)	(+ / -)	(✓)	(+ / -)	(✓)	(+ / -)	(✓)	(+ / -)	(✓)	(+ / -)	(✓)	(+ / -)	(✓)	(+ / -)	(✓)	(+ / -)				
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Explanation to transition phase progression checklist

(✓) - The step is offered to the pig
(+ / -) The pig has either performed the desired step (+) Or not performed the step (-)
** - Three minutes in total to cover the steps during feeding. If the pig responds positively to the first action, the next action is offered. The steps during feeding are designed so they can be performed in one smooth progression.

Descriptions to each activity

Apples on the floor (2 min)	Half an apple prepared in treat slices is gently tossed into the pen, and the time i started. The pig is observed for two minutes to. If the pig eats apples within those two minutes (+) and if it does not (-)
Apples from the hand (2 min)	Half an apple is prepared and brought into the pen and the time is started. The caretaker sits just inside the gate and offers the pig a slice of apple from the hand. During two minutes if the pig takes the apple (+) and if it does not (-). When the pigs understand this step they will quickly eat the apples. When the all the slices are used the caretaker steps outside the pen. The caretaker remains calmly just outside the pen for the rest of the time.
The pig accepts being touched during feeding**	During feeding the caretaker squats down next to the pig. A hand is gently extended toward the pig. If the pig backs away from the touch (-) if it accepts the touch (+). If the touch is accepted the caretaker can slowly try to stand up. Three minutes are allocated to the 3 actions during feeding, so if the time allows it the next action is initiated. If the pig backs away the hand is withdrawn slowly and the caretaker remains inside the pen for the rest of the time.
During feeding, touch ears and neck**	During feeding the pig accepts being touched on the neck and ears (+) If it moves away (-)
During feeding, touch hind and tail**	During feeding the pig accepts being touched on the hind and the tail (+) If it moves away (-)
With treats only, touch ears and neck	Two caretakers. Caretaker 1 stands outside the pen and gives treats to the pig through the bars. Caretaker 2 is inside the pen and touches the pig on the ears and neck (+) If it moves away (-). This is more challenging to the pig as it is fully aware of its surroundings. It mimics the situation of sample collection from the ears or injection in the neck area.
With treats only, touch hind and tail	Two caretakers. Caretaker 1 stands outside the pen and gives treats to the pig through the bars. Caretaker 2 is inside the pen and touches the pig on the hind and tail (+) If it moves away (-). This is more challenging to the pig as it is fully aware of its surroundings. It mimics the situation of taking a rectal temperature.