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# Vaccine safety testing using magnetic resonance imaging in suckling pigs

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ABSTRACT

Safety testing is one major part of the licensing procedure for veterinary vaccines and demands a large number of animals. Magnetic resonance imaging (MRI) was tested as an alternative, which may lead to a reduction in numbers of animals required for safety testing, and, correspondingly to a detailed description of the three-dimensional extent of the local tissue reaction repetitively in live pigs. In previous pig studies the following questions arose:

(1) Can MRI be used in suckling pigs in terms of safety testing?
(2) Does the injection of 2 ml saline solution lead to a volume effect resulting in tissue alterations comparable to a vaccine response?
(3) Is the local reaction size affected by the tattoo marking of the injection point for the final pathomorphologic examination?

To answer these questions the following study was performed by comparing two vaccine groups of suckling piglets (8 animals per group; A and B) with two control groups (4 animals per group; C and D). One control group was injected with a saline solution (C) and the other was only tattoo marked (D). The animals were examined using MRI at days 1, 8, 15, 22, 29, 36, and 43 post vaccination, ending with a final pathomorphologic examination. Pathomorphologic examination confirmed MRI findings. Saline solution does not result in a local tissue reaction as detected after injecting vaccines. Tattoo marking causes no local tissue reaction, neither in MRI nor in pathomorphologic examination. Therefore, MRI can be used as an alternative method for safety testing of vaccines in pigs of different age categories offering repetitive measurements of local tissue reactions. Involved cells might be examined only in a final pathomorphologic examination at the end of the trial on a reduced number of animals.


## 1. Introduction

Safety tests are required for the licensing procedure of veterinary vaccines and include the evaluation of the local tissue reaction [1,2]. In farm animals, vaccination is one of the most important interventions to prevent disease outbreaks, reduce infection rates, and therefore aid in the reduction antibiotic drug use [3]. Many farm animals are vaccinated several times throughout their lives against a large number of potential diseases. In pig farming, piglet vaccination has been becoming more and more important.

Killed vaccines usually contain an adjuvant which modulates and enhances the immune reaction towards the antigen of the vaccine. It is well known that the adjuvant used can impact the size and kind of local reaction, and therefore the choice of adjuvant affects the tissue lesion [4–8]. As most swine vaccines contain an adjuvant, an inflammatory reaction at the injection site has to be expected [9–11].

In previous studies [12,13] magnetic resonance imaging (MRI) was used to repetitively evaluate local tissue reactions post vaccination in pigs. Both previous studies evaluated the local tissue reaction in pigs of different age groups after injection of licensed vaccines using MRI. Differences between vaccine responses and progression courses were detected. A high relationship between final pathomorphological examination of the injection site and MRI results was found. Resulting in the conclusion, that the use of MRI as a noninvasive method could reduce the number of

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animals required according to the 3R principle of Russell & Burch [14], and can describe the local tissue reaction in three-dimensional extent. Though, some questions remained still open:

1. Can an open low-field MRI be used in suckling pigs to reliably detect vaccine response? In both previous studies [12,13], examination was performed in elder pigs, and because these pigs had a higher weight (10–87 kg at first examination day) they were easier to handle in terms of anesthesia and wake up time. Suckling pigs, which are the most sensitive group, have a lower weight and are more vulnerable in performing anesthesia than the animals tested previously by MRI *in vivo*. To evaluate whether the former used open low-field MRI is able to acquire acceptable MR images, suckling pigs with a weight (at first examination) below 10 kg were used in the present study. Additionally, vaccines were selected, which are representative for this age group, like a porcine Circovirus vaccine combined with *Mycoplasma hyopneumoniae*.
2. Does a tattoo, which marks the injection point for the final pathomorphologic examination, influence the local tissue reaction? This question came up already in both previous studies [12,13], which advised the need of marking the injection point, in order to be able to identify the injection point in a final pathomorphologic examination. Therefore, the question occurred, whether marking affects the local tissue reaction size post vaccination due to a former local inflammation in the skin based on the tattoo mark.
3. Does an injection of saline solution generate a local tissue reaction solely based on the volume effect similarly to an inflammatory reaction post vaccination? This could not be answered reliably in both previous studies [12,13] due to the lack of a control group. But some hints exist. Although the injection volume was always 2 ml [12,13], local tissue reaction sizes regarding the maximum extent combined with the examination day varied depending on the age group, and the vaccine used.

The present study was used to answer these questions.

## 2. Animals and methods

### 2.1. Management

The study was conducted in a commercial like setting of swine facilities. The experimental set-up including housing, feeding, and animal care taking was approved by the District Government of Upper Bavaria (registration number: 55.2-1-54-2532-87-2015), and followed local and national guidelines for animal experiments [15–17].

### 2.2. Animals

The animals (n = 24; 8.02 ± 1.35 kg live weight at first examination) were assigned to four different groups (A, B, C, and D; Table 1). Group A and B were vaccinated with one of the two combined vaccines. Group C and D served as control groups. Animals of group C received only an injection of saline solution, whereas animals of group D received no injection at all. The injection volume amounted to 2 ml in all cases (A–C; Table 1).

The piglets were crossbred offspring from Piétrain sires and German Landrace sows, randomly divided into experimental groups taking into consideration litter assignment. All piglets were born and raised at the swine facility. They grew up and were treated like all other pigs. At an age of four weeks, piglets were weaned and moved to a nursery deck where they were housed in groups of 15–22 animals (0.65–0.75 $m^2$ per animal). All boxes were enriched with balls, metal chains, and straw racks as required in the German Animal Welfare Regulation for Livestock Housing [16] to satisfy the exploration drive of the animals. All pigs were fed with an age specific diet (11–13 MJ/kg of ME age-related).

### 2.3. Sample size

Each vaccination group (A, B) consisted of 8 animals. For sample size calculation the GPower software package 3.1.6 [18,19] with an assumed power of 0.8 and an effect size of 1.25 was used. The sample size calculation for a matched paired t-test resulted in n = 8.

### 2.4. Testing scheme

At the 7th day of life, the injection point was marked with a circle (diameter 0.3 cm) on the skin of the left neck behind the ear in all 24 animals using a tattoo needle covered with black tattoo ink. This was performed to reliably identify the vaccination site at the final pathomorphologic examination. At an age of 25 days, all piglets of group A, B, and C were injected with either a vaccine or saline solution into the middle of the tattoo circle (day 0) using a sterile single-use needle (21 G × 5/8″; 0.8 × 16 mm). Before injection the health status of each animal was checked. No injection was performed into one of the animal's neck before day 0, so all animals were test and drug naïve. Post vaccination the animals were scanned using MRI in a weekly interval until day 43 (not all groups on all examination days). The examination procedure was stopped for group A and B at day 43. No reaction was detected in group C and D at day 8, therefore the imaging procedure was stopped for C and D at that examination day. Although imaging of group C was stopped at day 8, the final pathomorphologic examination was performed at day 43 in order to be able to compare all injected animals at the same point of time (Fig. 1).

### 2.5. Magnetic resonance imaging

All animals had to be anaesthetized for MRI scanning, using an intramuscular injection of Azaperone (Stresnil®, Janssen; 2 mg/kg body weight) and Ketamine (Ursotamin®, Serumwerk Bernburg; 20–40 mg/kg body weight), given into the muscles of the hind leg to avoid artifacts in the neck region. Before injection of anaesthetics, the health status of each animal was checked.

**Table 1**
Description of the animals, divided into the group with number of animals, gender and description of the injection ingredients and injection volume.

| Group | Number | Gender | Injection ingredients | Volume [ml] |
|---|---|---|---|---|
| A | 8 | 4 ♀ & 4 ♂ | Porcine circovirus type 2 ORF2 subunit antigen, *Mycoplasma hyopneumoniae* inactivated, Carbomer | 2 |
| B | 8 | 4 ♀ & 4 ♂ | Porcine circovirus type 2 ORF2 subunit antigen, *Mycoplasma hyopneumoniae* J strain inactivated, light mineral oil, aluminium (as hydroxide) | 2 |
| C | 4 | 2 ♀ & 2 ♂ | Saline solution 0.8% | 2 |
| D | 4 | 2 ♀ & 2 ♂ | – | – |