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# Magnetic resonance imaging to detect local tissue reactions after vaccination in sheep in vivo

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## ABSTRACT

**Objectives** Vaccination is one of the most effective methods to keep up the health status in humans and in livestock. Therefore, farm animals are vaccinated several times during their lifetime. Although vaccines are being checked regarding their local reactogenicity, side effects occur frequently—especially in the case of the application of adjuvanted products. Many reports exist about local reactions in sheep. The present study aimed at testing MRI as a method to document injection site reactions three-dimensionally.

**Design** Two groups of Merino lambs (n=16 each) were vaccinated subcutaneously into the left neck side. Two different, licensed inactivated vaccines were used. Both groups of lambs were anaesthetised and scanned using MRI at days 1, 3, 8, 15, 22 and 29 after vaccination.

**Setting** The study was performed on a commercial-like farm.

**Participants** Thirty-two Merino lambs entered the experiment, 16 male and 16 female ones (one animal died at day 22 after vaccination). At first examination day they were approximately three months old.

**Primary and secondary outcome measures** Volume differences were measured between vaccination and control neck side to evaluate the time pattern of local tissue reactions.

**Results** Local tissue reactions were visible on the skin surface and also appeared in deeper tissue layers on MRI. These deeper reactions would not have been found without MRI or, alternatively, without sacrificing the animals. Some of these extensive local reactions lasted for more than 29 days.

**Conclusions** The in vivo MRI results proved suitable to record local tissue reactions in terms of three-dimensional extent over a longer period of time in large farm animals without the need to sacrifice test animals. A three-dimensional MRI examination of the injection site during regulatory licensing studies offers an objective evaluation that could be used in a benefit-risk assessment of veterinary vaccines.

**Trial registration number** District Government of Upper Bavaria:55.2-1-54-2532-2-13.

## INTRODUCTION

Veterinary vaccines are an essential tool to prevent disease outbreaks, and to avoid negative effects on human health and animal production.[1–5] Effective vaccination schemes can reduce infections and therefore the use of antibiotic drugs, which is presently of major concern.[6]

A large number of farm animals kept for human consumption are usually vaccinated a couple of times against a large number of potential diseases throughout their life. Although vaccines are tested for their local reactogenicity, local side effects are being reported frequently, particularly after the use of adjuvanted products.[7–9] In sheep, most of these reactions are being camouflaged by the fleece and, therefore, becoming visible to farmers on a random basis.[7] However, such lesions negatively affect the carcass quality due to abscesses and could lead to concern at the slaughterhouse.[10]

In order to evaluate local tissue reactions in the live animal, imaging methods could be used. MRI, for example, is an approved method in human and companion animal medicine to study or to follow up soft tissue alterations and especially muscular disorders.[11–16] Musculoskeletal MRI studies are in most cases limited to pets or laboratory animals in terms of animal models.[17–19] While clinical studies in food-producing animals are rare, it has been shown in pigs that MRI can be used to record local tissue reactions, repetitively and in a three-dimensional extent.[20 21]

Various pathological conditions result in signal intensity changes in MR images. The incidence of alterations in muscle tissue signal intensities depends on the type of MRI sequence used and on the pathological condition being studied.[22 23] A variety of pathological conditions can be detected using different MRI protocols.[24 25]

Based on existing reports about local tissue reactions and the summary of product characteristics (SPC) of commercial sheep vaccines, this study aimed to evaluate and document local tissue reactions after vaccination in Merino lambs using MRI to provide

three-dimensional information about the local reactogenicity of sheep vaccines.

## MATERIALS AND METHODS

### Animals and management

A total of 32 Merino lambs (divided into two experimental groups) were used in the present study. Each experimental group consisted of 16 animals. The GPower software package V.3.1[26 27] served as tool for statistical planning of the trial. A power of 0.8 and an effect size of 0.66, which correlates with a large effect size, was used for sample size calculation resulting in n=16. The experimental set-up including housing and feeding was conducted in accordance with the District Government of Upper Bavaria (registration number: 55.2-1-54-2532-2-13) and was in compliance with local and national guidelines.[28–30]

Lambs were raised under conventional conditions of sheep farming. They were housed separately from other sheep in groups of eight lambs. Each group was housed in a 12 $m^2$ pen with concrete flooring and straw bedding. All lambs were fed with a diet containing 12 MJ/kg of metabolizable energy (ME). Hay, mineral supplement and water were provided ad libitum. The lambs were approximately three months old and had a mean (±sd) bodyweight of 49.3 (±6.3) kg on the first examination day without restriction of feed. These lambs had not received any vaccination prior to the experiment. The health status of all animals was checked by a veterinarian before the start of the experiment. None of the animals showed clinical signs of disease.

### Vaccination procedure

Each experimental group was vaccinated according to the manufacturer's instructions of the special product. Table 1 informs about both groups. Both vaccines used are licensed as well in Europe and other parts of the world.

Vaccination was performed on the left side of the neck (vaccination side; VS). The injection point was marked by trimming the region of injection in order to identify the vaccination point throughout the study. Each lamb was separated from the group during vaccination. A skin fold of the shaved dry neck region was raised and the vaccine was injected into the centre of this region. Each injection was administered using a sterile, single-use needle.

### MRI

Animals were examined using an open low-field MRI system (Siemens Magnetom Open; magnetic field strength 0.2 T) six times at days 1, 3, 8, 15, 22 and 29 after vaccination. The MRI unit was located at the farm where the sheep were housed.

T1- and T2-weighted MR image sequences were used to detect possible tissue abnormalities (such as haematoma, oedema, inflammation or fatty infiltration). The examination started with a T1-weighted spin echo sequence with a coronary image acquisition direction ($T1_c$), followed by a T2-weighted spin echo sequence with the same direction of image acquisition ($T2_c$). Table 2 displays the MRI acquisition parameters.

The MRI examination of the lambs took place under general anaesthesia using xylazine (Proxylaz, Veyx; 0.22 mg/kg, given intramuscularly) followed by ketamine (Ursotamin, Serumwerk Bernburg; 2 mg/kg, given intravenously). Xylazine was injected into the muscles of the hind leg, in order to avoid interactions with the local tissue reaction at the VS. Anaesthesia was maintained for 25 minutes by an uninterrupted application of a glucose (5%) dilution (Glucose 5%, B. Braun Melsungen AG; 4 ml/minute constant infusion) mixed with 2 mg ketamine per millilitre[31] into the ear vein.

The Able 3D Doctor Software (Food and Drug Administration approved; Lexington, MA, USA) served as tool for MR image analysis using the *region of interest (ROI)* function. Volumes of regions with increased signal intensity at the VS were compared with the control side (CS = right side of the neck). The *ROI* (see box in Fig 1) covered the largest extent of the area that showed increased signal intensity at the VS. The *interactive segmentation* function was used to define different tissue classes by separating them according to threshold settings for the measured

**TABLE 1:** Description of the two experimental groups (C, F) with number of animals, gender, weight at first examination day (as mean ± sd), the vaccine composition and the injection type and volume

| Group | Number | Gender | Weight* (kg) | Vaccine composition | Injection type and volume |
|---|---|---|---|---|---|
| C | 16 | 8 ♂<br>8 ♀ | 50.3±7.00 | *Clostridial* species potassium aluminium sulphate, thiomersal and formaldehyde | Subcutaneous, 5 ml |
| F | 16 | 8 ♂<br>8 ♀ | 48.3±5.65 | *Dichelobacter nodosus* light mineral oil NF, mannide oleate, thiomersal and formaldehyde | Subcutaneous, 1 ml |

*At first examination day.

**TABLE 2:** MRI protocols for the different used sequences

| | T1 | T2 |
|---|---|---|
| Pixel size (mm) | 1.30 x 0.70 | 1.43 x 0.78 |
| Examination time (seconds) | 340 | 348 |
| Time to repeat (ms) | 814 | 5690 |
| Time to echo (ms) | 17 | 102 |
| Slice number | 22 | 22 |
| Slice thickness (mm) | 4 | 4 |
| Acquisition direction | Coronary | Coronary |
| Distance factor | 0.50 | 0.50 |
| Matrix size (pixel) | 138 x 256 (54%) | 140 x 256 (55%) |
| Field of view ($mm^2$) | 180 x 180 | 180 x 180 |

T1, T1-weighted spin echo sequence; T2, T2-weighted spin echo sequence.

signal intensities on a grey scale level from 0 (black) to 4096 (white). Regions with increased signal intensities or grey values close to white were classified as hyperintense regions. Within the same image, the *ROI* of the VS was mirrored to the CS and the *interactive segmentation* function was applied again using the same thresholds for the signal intensities as on the VS. Ten images from each sequence, starting at the injection site, were recorded to create the final volumes of interest.

### Statistical analysis

SAS V.9.3 (SAS Institute, Cary, NC, USA) served as software basis. Animals served both as control and as treatment groups at the same time. Repeated measures analysis of variance (RM ANOVA) was used to assess the effect of experimental group (vaccine), day of examination and body weight on day of examination on the mean volume differences (vol_diff) of VS and CS using the generalised linear model (GLM) procedure of SAS. The repeated measures effect was the individual animal (animal number) and all two and three-way interactions were also tested. Under the assumption of normal distribution and violation of sphericity, the Huynh-Feldt epsilon and the corresponding adjusted P value have been enhanced to include a correction based on Lecoutre.[32] The level of significance was set at P≤0.05.

## RESULTS

MRI allowed a three-dimensional evaluation of the total extent of the local reaction (Fig 1) and a definition of the affected structures (subcutaneous vs. superficial muscle tissues).

The mean vol_diffs (least squares means ± standard errors of estimation) for the two vaccination groups (C and F) are shown for the T1-weighted (Fig 2a) and T2-weighted (Fig 2b) sequences; RM ANOVA showed that the vol_diffs in group F were significantly higher than in group C at days 8 (P=0.0208), 15 (P<0.0001), 22 (P<0.0001) and 29 (P<0.0001) for T1, and only at day 8 (P=0.0102) for T2 (see Tables 3 and 4 for the results of the F test).

Referring to the T1-weighted MRI data (Fig 2a), both groups started with an equal vol_diff at day 1, but resulted in different maximum local reaction volumes: group C with a maximum vol_diff of 4.9±1.5 $cm^3$ at day 15 (P=0.0016) and group F with 16.5±1.5 $cm^3$ at day 22 (P<0.0001). Both groups showed a decrease in vol_diff

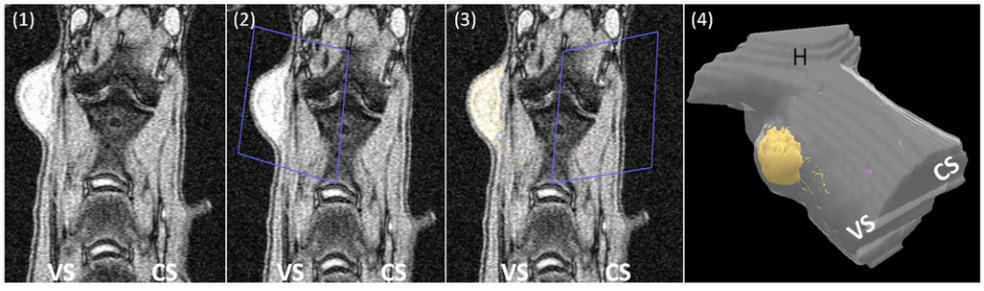


**FIG 1:** Evaluation of the increased signal at VS and CS. (1) A coronary T1-weighted MR image ($T1_c$) taken on examination day 29, showing a hyperintensive region at VS (group F, animal no. 27). (2) The same image with the ROI drawn around the largest extent of the hyperintense region on VS. (3) The same image showing the mirrored ROI on the CS, selecting tissues with the same signal intensity as on VS. (4) A three-dimensional reconstruction of the evaluated lamb neck, representing the volume of local reaction (yellow) on VS. CS, control side; H, head; ROI, region of interest; VS, vaccination side

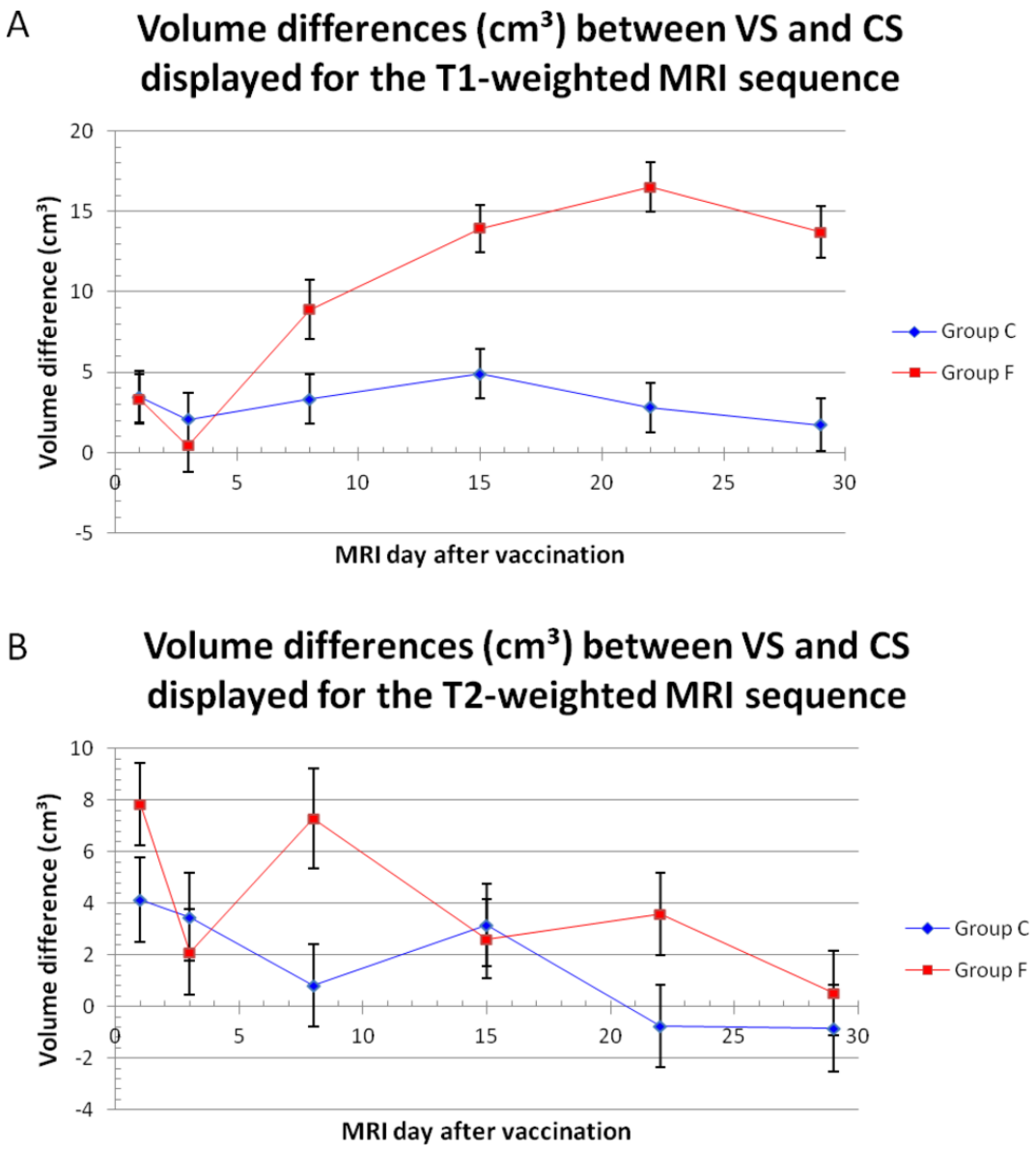


**FIG 2:** Results of repeated measures analysis of variance showing the calculated volume differences as vaccination side-control side (presented as volume difference) in $cm^3$ for the T1-weighted coronary sequence (A) and the T2-weighted coronary sequence (B). Group C was vaccinated against *Clostridial* species and group F was vaccinated against *Dichelobacter nodosus*. At day 29 after vaccination, in group F only 15 animals were examined, because one animal died after day 22

after reaching the maximum extent. The graph of the T2-weighted MRI sequence (Fig 2b) showed differences between both groups, regarding their estimated vol_diffs, and in the progression of vol_diffs. Group F showed a nearly eight times higher vol_diff (7.9±1.6 $cm^3$, P<0.0001) than the injection volume (1 ml), whereas group C showed a lower vol_diff (4.1±1.6 $cm^3$, P=0.0131) than the injection volume (5 ml) at day 1 after vaccination.

From a macroscopic point of view, some animals showed grossly palpable masses at the injection site, whereas in other animals the masses were fistulated (Fig 3). In group C, 6 out of 16 animals showed clearly visible

**TABLE 3:** Results of the generalised linear model analysis of variance for T1

| Source | F value | P value |
|---|---|---|
| Experimental group (vaccine) | 47.13 | <0.0001 |
| Day of examination | 9.10 | <0.0001 |
| Experimental group x day of examination | 8.11 | <0.0001 |
| Covariable bodyweight | 3.91 | 0.0499 |

**TABLE 4:** Results of the generalised linear modelanalysis of variance for T2

| Source | F value | P value |
|---|---|---|
| Experimental group (vaccine) | 5.75 | 0.0177 |
| Day of examination | 3.16 | 0.0095 |
| Experimental group x day of examination | 1.65 | 0.1510 |
| Covariable bodyweight | 0.39 | 0.5337 |

masses at VS (in one animal it was fistulated), whereas the other 10 animals showed thickened skin which was slightly bulged. In group F, 8 out of 16 animals showed clearly visible masses at VS and 50% of these were fistulated. The remaining animals of this group also showed thickened skin.

Using MRI, an examination of the affected tissues was possible at the living animal: more than 60% (10/16) of group C animals showed a local reaction in the subcutaneous and superficial muscle layers at day 15 after vaccination (the day of maximum extent of local reaction in group C), while less than 40% (6/16) of group F animals showed a local reaction in the subcutaneous and superficial muscle layers at day 22 after vaccination (the day of maximum extent of local reaction in group F).

## DISCUSSION

Both vaccines caused extensive local tissue reactions, although a sterile needle was used in each case and the vaccination site was trimmed and dry. These observations confirm what has been described in the SPCs of each vaccine and were reported in other studies with clostridial or footrot vaccines.[7 33–36] Published studies[37 38 7] stated that subcutaneous swelling with a diameter of up to 5 cm seems to be a normal reaction in clostridial vaccination. Therefore, it seems that rather large local tissue reactions after vaccination are being tolerated in sheep. These large local tissue reactions, however, might become an animal welfare issue, as these reactions are likely to be painful and cause distress in affected sheep. The present study confirms former reports about long-lasting (29 days after vaccination) and extensive (5–15 $cm^3$ vol_diff) local reactions after vaccination in sheep. Several reports exist about vaccination site reactions in sheep which describe extensive local tissue reactions throughout a longer period of time from up to 120 days[34] to nine months after vaccination.[37] Local reactions could result in scar tissue, fibrosis or solid abscesses due to cell death (necrosis) of tissue at the injection site and this could negatively affect the carcass quality and result in objections at the slaughterhouse as published by Eppleston and Windsor.[10]

Due to these findings, we suggest that the current requirement of an examination period of 14 days after vaccination is not long enough to evaluate the total extent of any local tissue reaction in the licensing procedure for veterinary vaccines in terms of safety assessment. Local tissue reactions caused by vaccination in food-producing animals are important and should not be considered to be normal because of economic reasons and the animal welfare impact. Differences in local tissue reaction sizes should also be considered, as vaccination is an important method to control infectious diseases and to protect animals from suffering and pain due to sickness.[2 3 5] This could reduce the need of antibiotic drugs[6] which is of major concern nowadays with the increasing occurrence of multiresistant bacteria.

The MRI data from the present study allowed an exact three-dimensional determination of the local tissue reaction repetitively in live animals. The local tissue reaction showed increasing vol_diffs during the examination period reaching a maximum at day 15 (group C) or at day 22 (group F) after vaccination. The time pattern of group C (Fig 2a) confirmed the findings by Green and others.[7] They measured local tissue masses in sheep using a calliper at days 0, 1, 7, 14 and 28 after clostridial vaccination and found an increase in the extent of the local tissue reaction until day 14. Signal intensity changes in T1-weighted images in the present study are likely caused by inflammatory processes.[22 39] This was confirmed by the present study, showing the formation of subcutaneous masses with yellow caseous debris demonstrated in the sheep macroscopically.

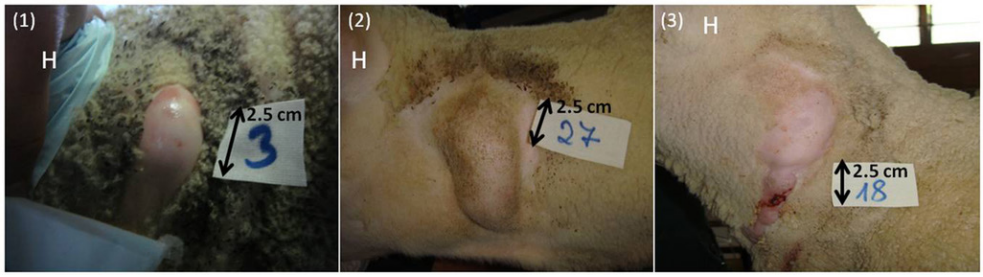


**FIG 3:** Photographs showing a subcutaneous mass at the vaccination side of three lambs: (1) animal number 3 of group C at day 22, (2) animal number 27 of group F at day 29, and (3) fistulated mass of animal number 18 of group F at day 22. H, head.

Previous studies about vaccination lesions in sheep describe subcutaneous masses at the injection site independently of the vaccine used.[33 37 38] Signal intensity changes in T2-weighted MR images represent tissue alterations caused by oedematous tissue variations, haematoma or fatty infiltration.[22 39 40] In the present study, we suggest that the signal intensity increase in the T2-weighted images likely reflects considerable inflammation resulting in oedematous tissue.

Both vaccines resulted in different maximum vol_diffs and different time patterns of reaction. Group F showed a more extensive and longer lasting local reaction with a three times larger maximum vol_diff than did group C (Fig 2). Two explanations are possible. The different time patterns may have resulted from either a difference in the volume injected or the use of different adjuvants in the two vaccines. If the extent of tissue reaction was related to the injection volume, the maximum volume of local tissue reaction should have been found on day 1 after vaccination. However, the maximum vol_diffs detected using the $T1_c$ sequence occurred on day 15 for group C and day 22 for group F after vaccination. Additionally, an effect due to the volume injected can be excluded in the present study since the maximum vol_diff was not found in group C where the injection volume was 5 ml, but was found in group F where the injection volume was only 1 ml (Table 1). Adjuvants also have an important influence on the local reaction as reported in previous studies.[33 41–44] Studies by Lambell[34] and Ross and Titterington[33] reported severe side effects after footrot vaccination. Ross and Titterington[33] showed that an oil-based vaccine results in larger lesions than alum-precipitated vaccine and our results agree with this.

In addition to the MRI data presented here, the macroscopic results showed that the local tissue reaction was easy to detect because the injection site was shaved. But, if a mass was fistulated, the extent of the local reaction could not be determined macroscopically. Green and others[7] reported that tissue reactions at the injection site frequently may be camouflaged by the fleece and are only detected at slaughter. Even if a mass is not fistulated, it may not be possible to evaluate damage in the underlying muscle tissue macroscopically. Eppleston and Windsor[10] reported negative economic impacts at slaughter if reactions at the injection site lead to a downgrading of the carcass value. MRI allows an evaluation of the whole three-dimensional extent of the local tissue reaction and an exact description of the affected soft tissues. Although the macroscopic view was not representative of the affected tissue at the injection site below the skin surface, the use of MRI made it possible to detect and follow the affected tissues under the skin surface by demonstrating individual differences for the local tissue reactions depending on the vaccine used. The MR images demonstrated that beside the subcutaneous mass development, superficial muscle tissues were affected by the local reaction in various degrees, which could not be detected via palpation or without sacrificing the animals. Therefore, MRI offers additional information in terms of safety assessment of veterinary vaccines and can be used to give non-invasive insights in the patient's body.

Our results suggest a need for changes to vaccine ingredients/adjuvants or in alternative and precise methods for the evaluation of local tissue reactions after vaccination before new products are licensed. We have shown that methods such as MRI are suitable methods for such purposes. MRI has a distinguished soft tissue contrast and offers the possibility to measure the total tissue reaction extent without affecting the injection site due to direct contact and therefore not modifying the object of interest. MRI allows follow-up examinations of live animals. Based on its technical background, it is very well suited for the detection of different tissue pathologies in extent and quality and this makes it an ideal method for supporting the licensing procedure of veterinary vaccines.

## CONCLUSIONS

Local tissue reactions detected in our study show the need for more precise methods during the licensing procedure of veterinary vaccines, in order to produce safe and effective vaccines by avoiding pain and distress related to large tissue reaction sizes.

In vivo MRI is suitable to detect, monitor and evaluate local tissue reactions in live lambs. A three-dimensional examination of the injection site of large farm animals during regulatory licensing studies offers an objective evaluation that could be used in a benefit-risk assessment of veterinary vaccines. Additionally, alternative adjuvants or routes of administration should be examined in order to guarantee a safe but effective vaccine or to describe the best way of drug injection. Alternatively, the development of new vaccines might focus on non-adjuvanted products in order to further improve the benefits of vaccination as important factor for human and animal health management.

**Contributor** MB: substantial contribution to conception and design, acquisition of data and analysis and interpretation of data; drafting the article; final approval of the version, PVK: substantial contribution to conception and design, acquisition of data; revising the article critically; final approval of the version, LSK: acquisition of data; revising the article critically; final approval of the version, SS: acquisition of data; revising the article critically; final approval of the version, KC: substantial contribution to conception; revising the article critically; final approval of the version, AH: acquisition of data; revising the article critically; final approval of the version, AMS: substantial contribution to conception and design; revising the article critically; final approval of the version.

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