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Infrared thermography used to detect local adverse reactions induced by reproductive vaccine adjuvants in Holstein heifers[1]

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ABSTRACT.- Baccili C.C., Santarosa B.P., Nichi M., Martin C.C., Ramos J.S., Decaris N., Benesi F.J. & Gomes V. 2024. **Infrared thermography used to detect local adverse reactions induced by reproductive vaccine adjuvants in Holstein heifers.** *Pesquisa Veterinária Brasileira 44:e07507, 2024*. Departamento de Clínica Médica, Faculdade de Medicina e Zootecnia, Universidade de São Paulo, Av. Prof. Dr. Orlando Marques de Paiva 87, Cidade Universitária, Butantã, São Paulo, SP 05508-270, Brazil. E-mail: viviani.gomes@usp.br

Local adverse reactions following vaccination, often tied to the adjuvant type, can lead to pain, fever, reduced intake, and weight loss. Infrared thermography (IRT), a non-invasive human and veterinary medicine technique, can assess local inflammation. This study aimed to evaluate such reactions induced by reproductive vaccines with different adjuvants, using IRT alongside cardinal signs, rectal temperature, and haptoglobin concentration. Thirty-five Holstein heifers were grouped by vaccine type: Ah (aluminum hydroxide), Ow (oil-in-water), QAD (amphigen and quil A cholesterol and dimethyl-dioctadecyl ammonium bromide adjuvant), and a Control (saline solution). Assessments were made at 0, 6, 24-, 48-, 72-, and 168 hours post-vaccination for both doses, with an interval of 21 days. The local reactions were evaluated using the inflammatory cardinal signs and surface temperature measurement using IRT. The systemic reactions were identified by rectal temperature and the concentration of haptoglobin. A larger proportion of animals exhibiting local reactions based on scores assigned to the cardinal signs was found in the Vaccine QAD group, as well as the rectal temperature and the frequency of heifers with inflammation (Hp ≥2mg/dL). Nevertheless, Vaccine Ow demonstrated higher temperature at the site after the first vaccination dose for the IRT. Therefore, this approach is a valuable tool in classifying responses and local inflammation following vaccination in heifers with reproductive vaccines. Concurrently evaluating systemic manifestations, facilitates the surveillance of adverse reactions, thereby improving the discernment of the extent of systemic and local effects.

INDEX TERMS: Adverse effects, infrared thermography, dairy cattle, Holstein heifers, inflammation, infectious diseases, vaccination.

RESUMO.- [**Termografia infravermelha usada para detectar reações adversas locais induzidas por adjuvantes de vacinas reprodutivas em novilhas Holandesas.**] Reações adversas locais após a vacinação, frequentemente associadas ao tipo de adjuvante, podem levar a dor, febre, redução na ingestão e perda de peso. A termografia infravermelha (IRT), uma técnica não invasiva utilizada na medicina humana e veterinária, oferece um meio de avaliar a inflamação local. Este estudo teve como objetivo avaliar tais reações induzidas por vacinas reprodutivas com diferentes adjuvantes, utilizando IRT junto com sinais cardinais, temperatura retal e concentração de haptoglobina. Trinta e cinco novilhas Holandesas foram agrupadas por tipo de vacina: Ah (hidróxido de alumínio), Ow (óleo em água), QAD (anfígeno e adjuvante de quil A colesterol e brometo de amônio dimetil-dioctadecil) e Controle (solução salina). As avaliações foram realizadas às 0, 6, 24, 48, 72 e 168 horas pós-vacinação para ambas as doses, com um intervalo de 21

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dias. As reações locais foram avaliadas pelos sinais cardinais inflamatórios e pela aferição da temperatura superficial usando IRT. As reações sistêmicas foram identificadas pela temperatura retal e pela concentração de haptoglobina (Hp). Uma proporção maior de animais exibindo reações locais com base nos escores atribuídos aos sinais cardinais foi encontrada no grupo Vacina QAD, assim como na temperatura retal e na frequência de novilhas com inflamação (Hp ≥2mg/dL). No entanto, para a IRT, a Vacina Ow demonstrou temperatura mais elevada no local após a primeira dose de vacinação. Portanto, essa abordagem é uma ferramenta valiosa para classificar as respostas e a inflamação local após a vacinação em novilhas com vacinas reprodutivas. Avaliar simultaneamente as manifestações sistêmicas facilita a vigilância das reações adversas, melhorando assim o discernimento da extensão dos efeitos tanto sistêmicos quanto locais.

TERMOS DE INDEXAÇÃO: Efeitos adversos, termografia infravermelha, doenças infecciosas, gado leiteiro, gado Holandês, inflamação, vacinação.

INTRODUCTION

Investments in reproductive biotechnologies like fixed-time artificial insemination (FTAI) and embryo transfer (ET) may suffer losses due to infectious agents such as bovine viral diarrhea virus (BVDV), bovine herpesvirus type-1 (BoHV-1) (Walz et al. 2017), and Leptospira spp. (Fávero et al. 2018). These pathogens risk the success of these biotechnologies, potentially causing financial setbacks. They can induce abortions and lead to losses such as reduced conception rates, early embryonic death, premature births, stillbirths, and persistently infected calves. Preventive measures include vaccinating adult cattle, which produce higher concentrations of protective antibodies against these diseases (Aono et al. 2013, Pereira et al. 2013). Vaccines for infectious reproductive agents come in various formulations with live or inactivated antigens diluted in adjuvants (Vartak & Sucheck 2016). They are typically administered through subcutaneous or intramuscular injections, which may cause local post-vaccine reactions characterized by pain, heat, redness, and swelling (Wheater et al. 1985). These reactions trigger immunological events activating an acquired cellular immune response by T helper type 1 cells (Th1) and a humoral response (Th2) (Roth 1999).

Enhancing inactivated vaccines in larger quantities or using certain adjuvants can increase the risk of animals experiencing adverse local reactions, leading to discomfort after repeated administrations. These reactions, documented by Shams (2005), Newcomer et al. (2017), and Bamouh et al. (2021), can result in firm nodes at the injection site, impairing carcass classification scores and diminishing meat quality in international markets, as noted by Leal et al. (2014). Additionally, systemic reactions may occur, causing reduced food intake, rumination, and milk production, as observed by Robattini et al. (2020). Leal et al. (2014) estimated economic losses of approximately R$ 20,424.00 due to local reactions following foot-and-mouth disease vaccination in Brazil. George et al. (1995) found that injection-site lesions affected 9.74% of examined round cuts in the United States, averaging 211.8g of lesion-trim, resulting in over $9 million in annual losses for the beef and dairy industries (Roeber et al. 2002). However, they noted a decline in the frequency of injection-site lesions from 1998 to 2000. Furthermore, deaths have been linked to anaphylactoid or anaphylactic allergic reactions (George et al. 1995), emphasizing the need for rigorous quality control in the pharmaceutical industry to ensure both adequate immunity levels and minimal adverse reactions, as emphasized by Ridpath et al. (2010).

Infrared thermography (IRT) allows the observation of different aspects of thermal physiology, injuries, and disease identification. The great advantage of this technique is that it can be used as a non-invasive evaluation in animals and can be applied in different sectors of veterinary medicine (McManus et al. 2022). In cattle, it has been reported to study tuberculin reactions (Merkal et al. 1973), heat tolerance (Daltro et al. 2017), reproduction of bulls (Teixeira et al. 2019) and dairy cows (Perez Marquez et al. 2019), intramammary infections (Chakraborty et al. 2019), subclinical bovine mastitis (Oliveira et al. 2022), digestibility and methane emission (Ornelas et al. 2019), omphalitis (Shecaira et al. 2018), viral bovine diarrhea (Schaefer et al. 2004), and bovine respiratory disease (Schaefer et al. 2007). Cook et al. (2015) employed IRT to identify systemic reactions post-vaccination in pigs. Nevertheless, a notable gap exists in precise data correlating the application of IRT to identifying both local and systemic adverse effects following vaccination in cattle. Hence, the primary aim of this study was to examine local adverse reactions through thermography and inflammation indicators resulting from reproductive vaccination with commercially available vaccines encompassing varied adjuvant compositions. Additionally, the investigation assessed systemic reactions by monitoring rectal temperature and haptoglobin serum concentration.

MATERIALS AND METHODS

Ethical approval. This research was approved by the Animal Care and Use Committee (Approval number: 6229201216) of the "Faculdade de Medicina Veterinária e Zootecnia" (FMVZ) of the "Universidade de São Paulo" (USP).

Farm and dairy heifer vaccination. We included 15 to 24-month-old (19.4±4.02 months) Holstein heifers (n=35) from the Agency of Agribusiness Technology's dairy cattle herd located in the city of Nova Odessa, São Paulo State, Brazil (22°75' S latitude and 47°27' W longitude). The farm's health protocol consists only of the mandatory vaccines required by the "Ministério da Agricultura, Pecuária e Abastecimento" (Ministry of Agriculture, Livestock, and Supply – MAPA): Foot-and-Mouth Disease and Brucellosis (FAO 2017).

The field experimental stage was conducted from November 2015 to March 2016, spanning the summer and autumn seasons. The heifers were managed extensively with access to water and mineral salt *ad libitum*. Thirty-five heifers were selected, of which 68.57% (24/35) had not been included in artificial insemination (AI) protocols; 17.14% (6/35) were pregnant with a gestational age of around 90 days, and 14.28% (5/35) had been inseminated one month prior (October 5th, 2015) to the start of this research.

Data of day temperature (TEMP – °C) and relative humidity (RH – %) were obtained from the automatic meteorological station OMM:86868 located in Piracicaba/SP (22°42" S 47°37" W). They were provided by the "Instituto Nacional de Meteorologia" (Brazilian National Institute of Meteorology – INMET 2019). The field experiments were conducted from December 2015 to April 2016. The temperature and humidity index (THI) was estimated according to the equation proposed by Thom (1958): THI = [0.8 ×

TEMP + (RH/100) × (TEMP – 14.4) + 46.4]. The data is expressed in the Supplemental Table 1. The mean values during the first dose were 22.66±0.76°C of TEMP, 89.01±4.88% of RH, and 1,040±45.65 of THI. The second dose was performed after 21 days, and the mean values were 21.14±0.86°C of TEMP, 95.01±3.26% of RH, and 949.79±50.52 of THI.

The heifers (n=35) were randomized and distributed into four experimental groups based on the different adjuvant formulations in the vaccines: Vaccine Ah (aluminum hydroxide; n=9), Vaccine Ow (oil-in-water; n=10), Vaccine QAD (amphigen and quil A cholesterol and dimethyl-dioctadecyl ammonium bromide adjuvant; n=10), and the Control (saline solution; n=6) (Table 1). The vaccines were administered subcutaneously in the right side of the neck using a 120 × 40mm single sample needle (Precision Glide®, BD Diagnosis, Franklin Lakes/NJ, USA) and a 5mL syringe (Plastipak®, BD Diagnosis, Franklin Lakes/NJ, USA). During management, the vaccines and saline solution were stored in Styrofoam boxes with recyclable ice at the same refrigeration temperature. The heifers received two doses of the vaccines (5mL) at a 21-day interval, and the unvaccinated group (Control) received saline injections (5mL) at the same 21-day interval. The heifers were evaluated for rectal and skin temperature at 0 h and following the vaccination at 6, 24, 48, 72, and 168 h (Fig.1). Adverse effects were considered as manifestations exhibited by the heifers in the form of local and systemic reactions before and after the administration of the vaccines.

Local adverse reactions. Thermographic images were captured in a covered region inside the management pen. The location was not closed but there was no insolation. The timings for capturing the images were set from 10 p.m. to 12 p.m. for the evaluation before the vaccine administration and from 4 a.m. to 6 a.m. for the evaluations of any adverse reactions at the 6, 24, 48, 72, and 168-h timepoints post-vaccination.

It was determined that thermograms would be captured of the lateral aspect of the heifer's neck within an area of approximately 10 × 10cm and at a distance of 1m in a straight line along the right side of the animal's body. The camera that was used was an infrared camera, model FLIR T440 (FLIR Systems, Inc., Portland, USA), with an image frequency of 60 Hz, a spectral range of 7.5-13µm, a resolution of 640 × 480 pixels and thermal sensitivity of <0.045°C to 30°C; it was able to record an average temperature from -20°C to 1,200°C

Table 1. Vaccines with different antigens and adjuvants were used in Holstein heifers

Groups	Antigens
Vaccine Ah (*n*=9)	BVDV types 1 and 2 (inactivated) BoHV-2 (inactivated) *Campylobacter fetus, Campylobacter fetus subsp. venerealis, Leptospira interrogans serovar Pomona Histophilus somn*i
Vaccine Ow (*n*=10)	BVDV types 1 and 2 (inactivated) BoHV-2 and BoHV-5 (inactivated) *Leptospira hardjo*, *L. icterohaemorrhagiae*, *L. bratislava*, *L. pomona* and *L. wolffii*
Vaccine QAD (*n*=10)	BVDV types 1 and 2 (inactivated) BoHV-2 (thermosensitive) *Leptospira canicola*, *L. grippotyphosa*, *L. hardjo*, *L. icterohaemorrhagiae* and *L. pomona*
Control (*n*=6)	Saline solution

BVDV = bovine viral diarrhea virus, BoHV-2 = bovine herpesvirus; Base adjuvants: Vaccine Ah (aluminum hydroxide), Vaccine Ow (oil-in-water), Vaccine QAD (amphigen and quil A cholesterol and dimethyl-dioctadecyl ammonium bromide), Control (saline solution).

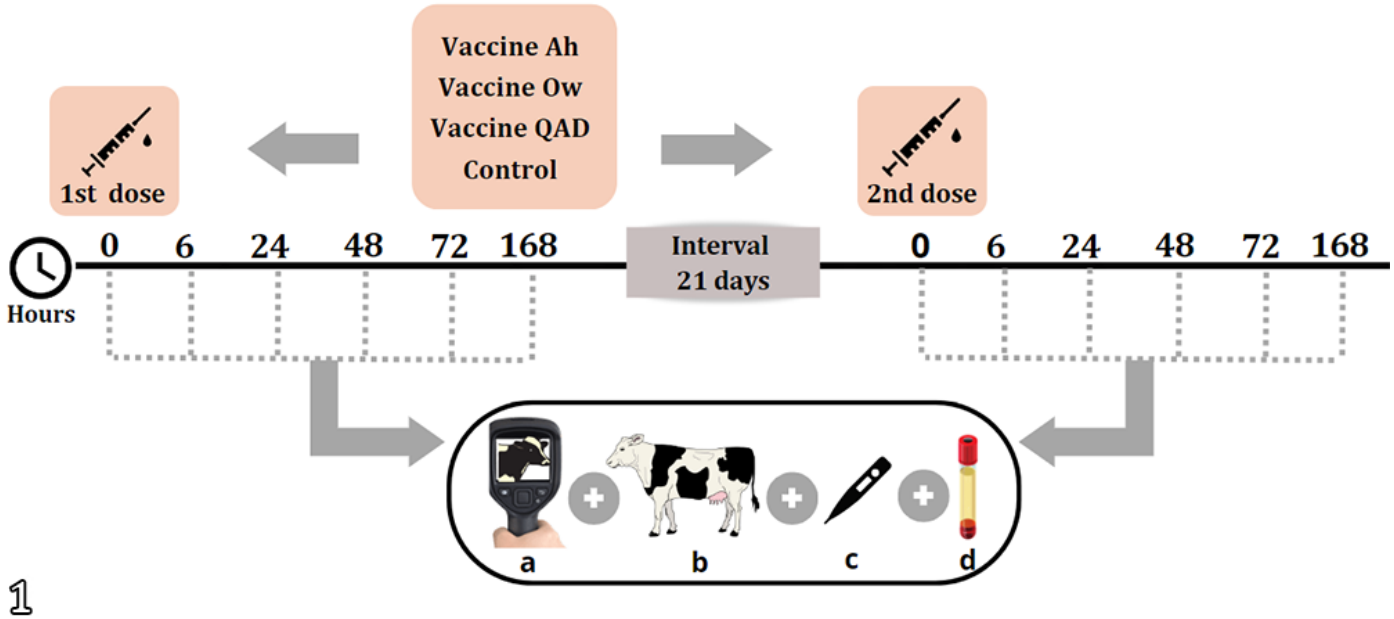

Fig.1. Graphical abstract of the timeline of experimental design.

and each degree was represented by a scale of different colors indicated at the bottom of the device and depicted in the captured image. The aforementioned thermographic camera automatically corrects environmental thermal differences and reflects emissivity to minimize the influences of the external environment.

The thermographic images were analyzed using a specific software program from FLIR Systems (FLIR Tools®) at an emissivity of 0.95%, a reflective temperature of 20°C, a relative humidity of 50%, and an atmospheric temperature of 20°C. In each thermogram, the injection site indicated by a red arrow was classified as having an area of 70mm^2. Within this area, the software program measured the maximum temperature (Tmax), minimum temperature (Tmin), and mean temperature (Tmean) in degrees Celsius (°C).

After the thermography, the heifers were evaluated by a periodical inspection and palpation of the injection site to detect local inflammatory reactions. The site reaction was evaluated by the detection of the cardinal signs of inflammation and was established a score to perform the evaluation: Zero for the absence (0); one for the presence of heat (1); two for pain (2); and three for redness (3). The number of animals that obtained heat, pain, and/or redness was added. Therefore, the final score ≥3 was used as the cut-off point to differentiate animals with or without local acute inflammation based on the cardinal signs.

Systemic adverse reactions. The rectal temperature of the heifers was measured with a digital thermometer (TH150®, G-Tech model, Shenzhen, China) and expressed in degrees Celsius (°C) in the range of 32.0 to 43.9°C before obtaining the thermographic images. Blood was collected from the heifers by jugular vein puncture. Blood was drawn into a vacuum tube without anticoagulant, which was allowed to clot at room temperature, and serum was collected by centrifugation at 2000 × g for 15 min, aliquoted, and stored at -20°C for three months or less after collection. Serum was used to haptoglobin levels at the Department of Internal Medicine of FMVZ-USP, São Paulo. The concentration of haptoglobin (Hp) was assessed using a turbidimetric assay based on the properties of Hp and meta-hemoglobin differential binding using the method described by Ramos et al. (2021). A cut-off value of 2.0mg/dL was used to classify the heifers as positive or negative for a systemic inflammatory response (Martin et al. 2021).

Statistical analysis. The data was analyzed using the Statistical Analysis System (SAS® version 9.4, SAS Institute, Cary/NC, USA). All the variables were evaluated for Gaussian distribution by a function-guided data analysis. Some data did not exhibit normal distribution and were subjected to a logarithmic transformation by log10, or the square or inverse square root was calculated to obtain a normal distribution of the variables.

The variables of temperature using infrared thermography were tested for the fixed effects of the treatments administered (Trt; Vaccines Ah, Ow, QAD, and Control) and for the various time points (0, 6, 24, 48, 72, and 168 h), as well as for the interaction between the effects of the treatment and hours (Trt x h) by a MIXED procedure (PROC-mixed, SAS) using the least significant difference post hoc test. The models were tested based on covariance structures using the Akaike information criterion. Differences were considered significant when $P \leq 0.05$.

Regarding the association with qualitative variables of cardinal signs (≥3) and Hp (≥2mg/dL), it was determined by Chi-square tests to analyze the group and inflammatory profiles at each time point of the study. The odds ratio (OR) with a confidence interval (CI) of 95% was also calculated (GraphPad InStat® Statistical software) to these parameters by comparing all the sample times (0, 6, 24, 48, 72, 168 h) after the first and the second dose of each Vaccine (Ah, Ow, and QAD) with the Control group.

RESULTS

Local reactions

The thermography temperatures (°C) measured at the injection site in Vaccines Ah, Ow, and QAD and the Control group, effects in each group at the different time points, and treatment interactions in the groups based on the time points were presented in Figure 2. The mean and standard deviation values of minimum, mean, and maximum temperatures were expressed in Supplemental Table 2. Figure 3 represents a

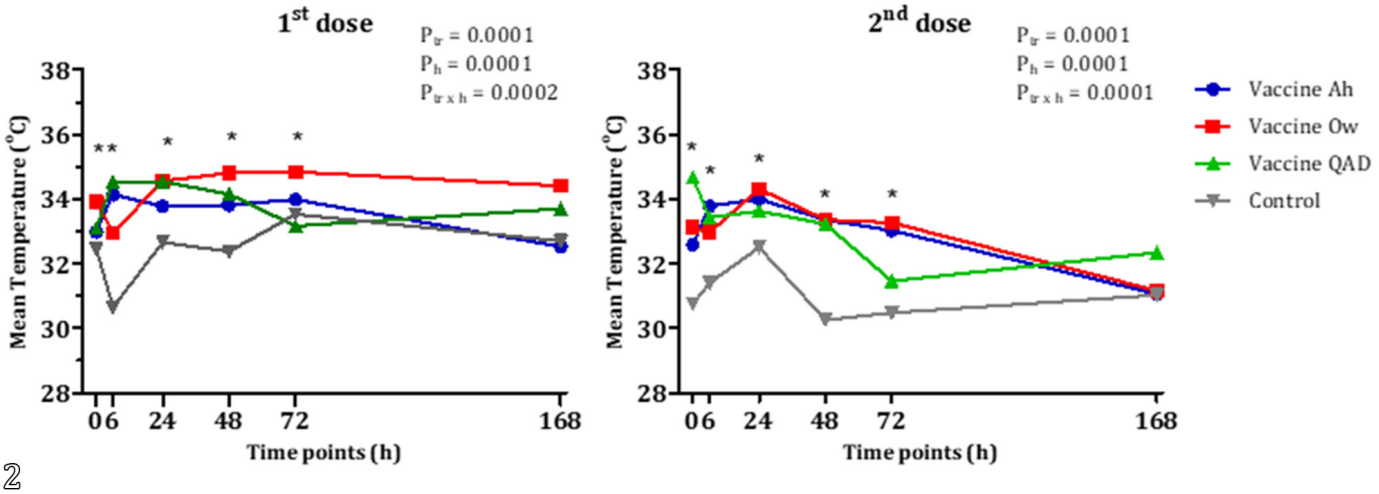

Fig.2. The mean temperatures were obtained by thermography of vaccinated heifers at the lateral neck region, comparing Vaccine Ah, Vaccine Ow, Vaccine QAD, and Control group after 1st and 2nd doses. Overall statistical significance ($P \leq 0.05$) is indicated in the plot as follows: Effects of treatment (Ptr), hours (Phours), and interactions (Ptr x hours). Difference among the treatments at each time point (asterisk) (Supplemental Table 2).

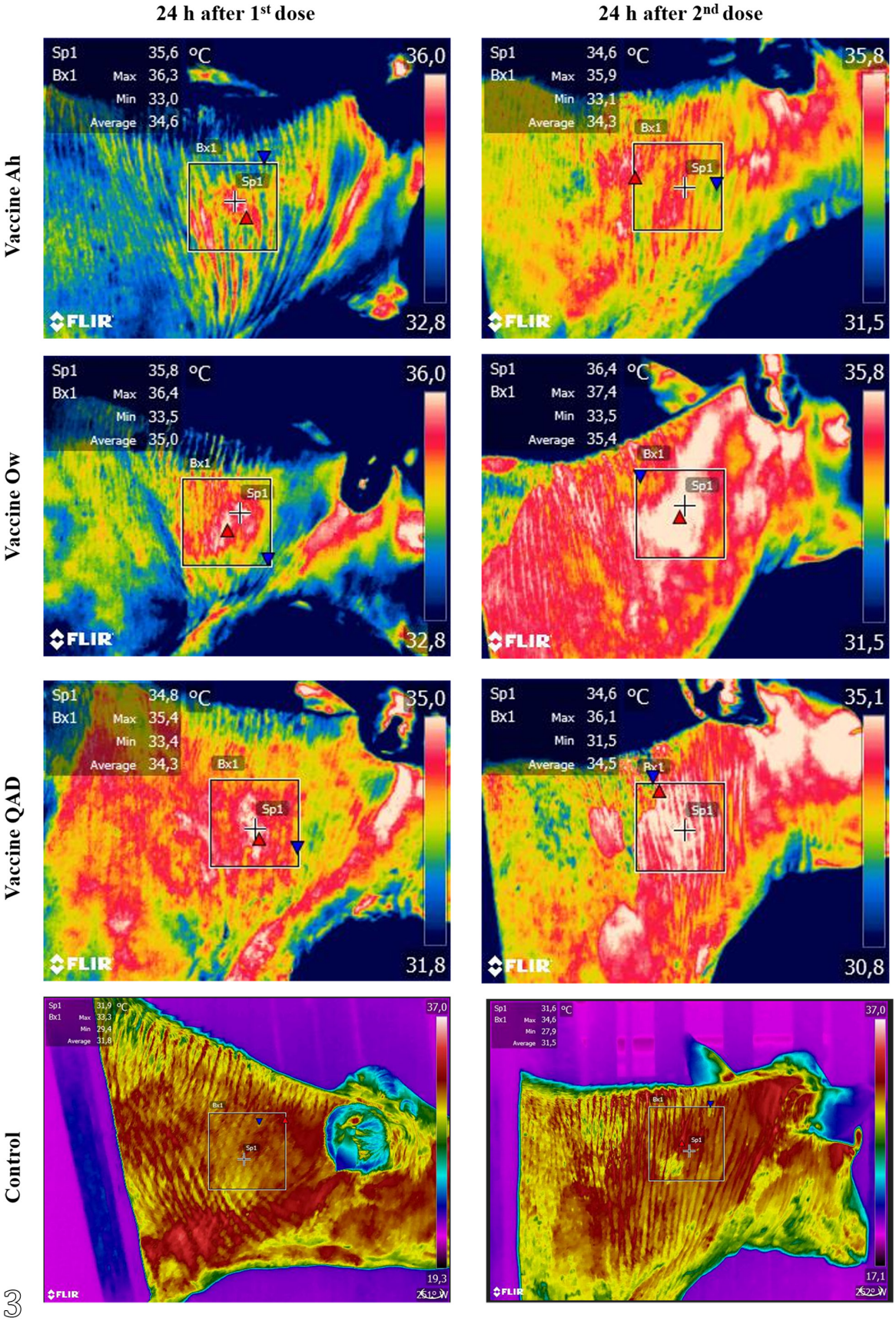

Fig.3. Thermographic image of the right lateral neck region of Vaccine Ah, Vaccine Ow, Vaccine QAD, and Control group at 24 h after 1st and 2nd doses. Maximum temperature site (red triangle). Minimum temperature site (blue triangle).

picture of different treatments 24 hours after the first and second doses of vaccination.

The Tmean exhibited the effects of the vaccine's formulations and times among the groups (P=0.0001; P=0.0001) and hours (P=0.0001; P=0.0001) and with the interactions of Trt × h (P=0.0002; P=0.0001) (Fig.2). The evaluated Tmean profile from 0 h to 72 h differed among the groups, exhibiting lower values in the heifers in the Control group concerning the Vaccinated groups. An increase in Tmean could be seen at the 6-h time point (34.14°C) for Vaccine Ah whereas it increased sequentially at the 24-h time point (34.56°C) for Vaccine Ow and remained elevated until 168 h (34.41°C). Vaccine QAD induced an increase in Tmean between 6 h (34.55°C) and 48 h (34.41°C), which decreased at 72 h (33.17°C). The highest temperature of 34.85°C observed in this study was induced by the oily formulation (Vaccine Ow) 72 h after the first dose.

On analyzing the thermal parameters observed following the second dose in the vaccinated heifers, an elevation in temperature was found at a later stage compared to the data described after the first dose. A higher Tmean was observed at 24 h following Vaccines Ow, Ah, and QAD, with mean temperatures of 34°C, 34.31°C, and 33.63°C, respectively. At the 72-h timepoint, the heifers vaccinated with Vaccine Ah (33.03°C) and Ow (33.27°C) presented with higher skin temperatures than the heifers vaccinated with Vaccine QAD (31.46°C) and the Control heifers (30.48°C).

Animals with local acute inflammation based on the cardinal signs are presented in Figure 4. A high frequency of positive animals was noticed at an earlier time point at 6 h (P=0.041) and 24 h (P=0.039) in the group of heifers receiving Vaccine QAD (70 e 60%), respectively, for the first dose. Vaccine Ah (77.8%) generated a profile of increasing the presence of cardinal signs with a peak at 72 h (P=0.011). For the second dose, the inflammation profiles of all the vaccines were similar, except for the 6 h (P=0.011) of Vaccine QAD (70%), which had a higher frequency of heifers presenting signs. No inflamed signs were observed in the Control group using the cardinal score in classifying them.

Considering the odds ratio (OR), all vaccines exhibited a higher likelihood of developing inflammation compared to the saline solution (Control group). Vaccine Ah presented the highest likelihood of exhibiting local inflammation signs after both the first (OR=50.53; 95% CI=2.94-867.37; P<0.0001) and the second dose (OR=46.85; 95% CI=2.72-804.70; P<0.0001). Simultaneously, Vaccine QAD also showcased elevated odds following both doses (OR=42.66; 95% CI=2.49-729.82; P<0.0001 after the first dose; OR=39.73; 95% CI=2.32-680.38; P=0.0002 after the second dose).

Nevertheless, the confidence interval (CI) after the initial vaccination with Vaccine Ow displayed a low likelihood of local inflammation despite the high OR (OR=16.96; 95% CI=0.96-297.40; P=0.0164). Conversely, following the second application of this vaccine, both the OR and the CI illustrated a high likelihood of local inflammation development (OR=22.76; 95% CI=1.31-394.72; P=0.0045).

Systemic reactions

A small frequency of vaccinated heifers showed only slight fever (≥39.5-40°C) by measurement of rectal temperature among the time points (Supplemental Table 3). Vaccine QAD caused slight fever in two heifers (20%) at 0 h and 24 h and three heifers (30%) at 6 h after the first dose. Otherwise, after the second dose, there was only one heifer (10%) at 0 h and 24 h and two animals (20%) at 6 h. Vaccine Ow caused only one heifer (10%) with a slight fever at 6 h after the second dose, while all the heifers vaccinated with Vaccine Ah had no fever at any time.

The frequency of animals exhibiting an inflammatory response based on haptoglobin above or equal to 2mg/mL expressed difference among the experimental groups (Fig.5). After the first dose, Vaccine QAD showed higher frequency at 24-h and 48-h compared to the others, as well as could be seen at 48-h after the second dose. Vaccine Ow had the highest frequency at 6-h after the second dose. Vaccine Ah resulted in only one heifer from 48-h to 168-h, while the Control group showed one animal at 168-h.

Further analysis revealed that Vaccine QAD resulted in a higher likelihood of stimulating the production of the acute-phase protein (Hp) after both the first dose (OR=15; 95% CI=1.90-118.11; P=0.0029) and the second dose (OR=16.96;

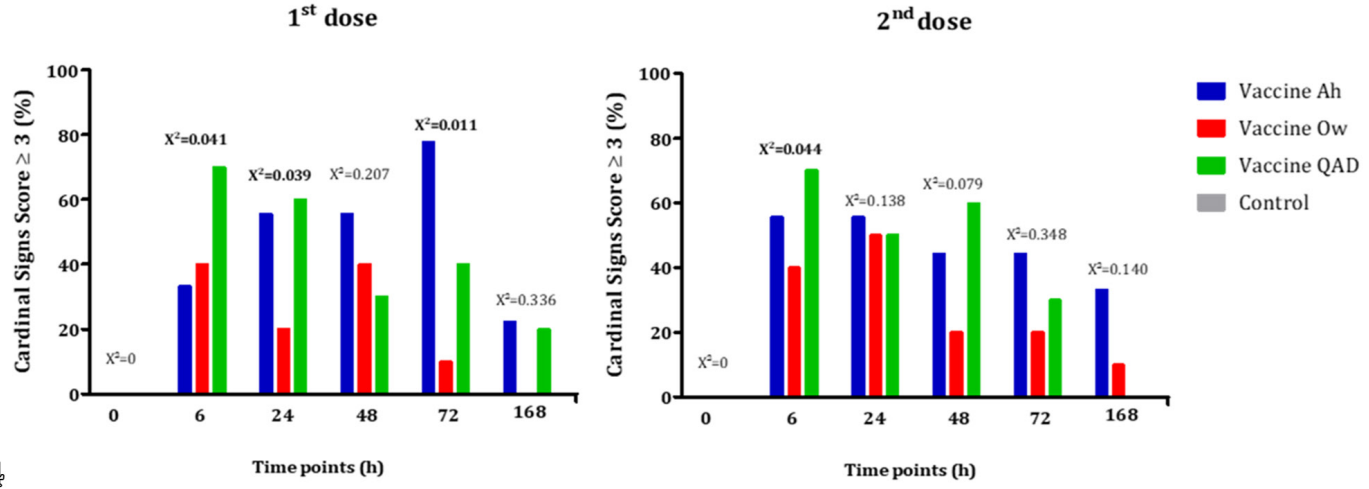

Fig.4. Frequency of vaccinated heifers comparing Vaccine Ah, Vaccine Ow, Vaccine QAD, and Control group with a score ≥3 based on cardinal signs after 1st and 2nd doses. X2 = Chi-square test. Differences were considered significant when P≤0.05.

95% CI=0.96-297.40; *P*=0.0164) compared to the saline solution (Control). Conversely, Vaccine Ow demonstrated a greater likelihood of causing systemic inflammation after the second dose (OR=20.74; 95% CI=1.19-360.89; *P*=0.0070). On the other hand, Vaccine Ah exhibited a lower likelihood of demonstrating a high concentration of Hp after the first dose (OR=2.05; 95% CI=0.20-20.63; *P*=0.9168) and none after the second dose.

DISCUSSION

In this research, IRT was employed to assess the local inflammatory profile induced by reproductive vaccines containing three different types of adjuvants. The description of local reactions, as well as the systemic reactions demonstrated by rectal temperature and Hp concentration, have already been published by the same authors (Baccili et al. 2019a). Additionally, Baccili et al. (2019b) discussed the humoral immune response linked to the effects of vaccination. Therefore, this study introduced the innovation by exploring the IRT data as an additional tool to detect local adverse reactions after vaccination.

The reactions observed through IRT varied after administering the first and second vaccine doses and among vaccines with different adjuvants. Following the initial dose, the three vaccines exhibited similar reactions from 0 to 6 h as marked by the Tmean of IRT. However, Vaccine Ow showed an increase of Tmean from 6 to 24 hours, and Vaccine Ah presented a higher reaction than Vaccine QAD at 72 hours. Throughout the other time points, Vaccine QAD displayed lower temperatures. After the second dose, Vaccine Ah and QAD exhibited similar temperatures from six to 72 hours, with a decrease from 48 to 72 hours. However, at 168 hours, Vaccine QAD had a higher temperature than the others. In general, a higher heat level was detected at the injection site in Vaccine Ow and QAD compared to Vaccine Ah and the Control group.

The action mechanisms of the different types of adjuvants are little known and described in the literature. Oily emulsions generally work via a depositing mechanism as they can form fat globes at the administration site of the vaccine (Xiang et al. 2006); these formations induce local apoptosis, cellular necrosis, and a major infiltration of leukocytes. The signals released by the apoptotic and necrotic cells attract phagocytes as well as dendritic cells or macrophages to the injection site, thereby resulting in higher inflammation levels (Shen & Yang 2012). This is why we observed a more intense reaction in the heifers that received the oily formulation (Vaccine Ow).

Exacerbated local stimulation is not a desirable (recommended) effect in bovine vaccination. Oily formulations are more reactive than alumni hydroxide and saponin adjuvants (Melo et al. 2019). Tissue damage caused by oils can be detected by local histological examinations, during which the following can be observed: Principally pyogranulomas, infiltrates of intact and necrotic neutrophils, and epithelioid macrophages with vacuolated cytoplasm, and externally, the abundant presence of lymphocytes and plasma cells amid the connective tissue (Leal et al. 2014). IRT revealed an earlier peak in the local temperature than the rectal temperature in the heifers vaccinated with Vaccine QAD at 6-h. In contrast, the heifers that received the Vaccine Ow (oily base) exhibited a peak at 24-h involving higher temperatures than those produced by the other vaccines until 168-h. Cook et al. (2018) evaluated animal welfare after the vaccination of piglets, and they used IRT. The authors observed elevated temperatures 24 hours after administering the vaccine, along with the animals clustering or gathering together in the pen. This fact corroborated the findings of the present study, which corresponded to the heifers that received the oily compound (Vaccine Ow). The increase in the local temperature suggested a stronger correlation between the physiologic alterations and immune challenges induced by vaccination (Cook et al. 2018).

A formulation containing amphigen/quil A cholesterol and dimethyl-dioctadecyl ammonium bromide (Vaccine QAD) is a potent complex immunostimulant that could produce a disseminated immune response, including a late-onset hypersensitivity reaction after repeated applications. A study previously published with the same experimental design (Baccili et al. 2019a), showed that vaccinated heifers

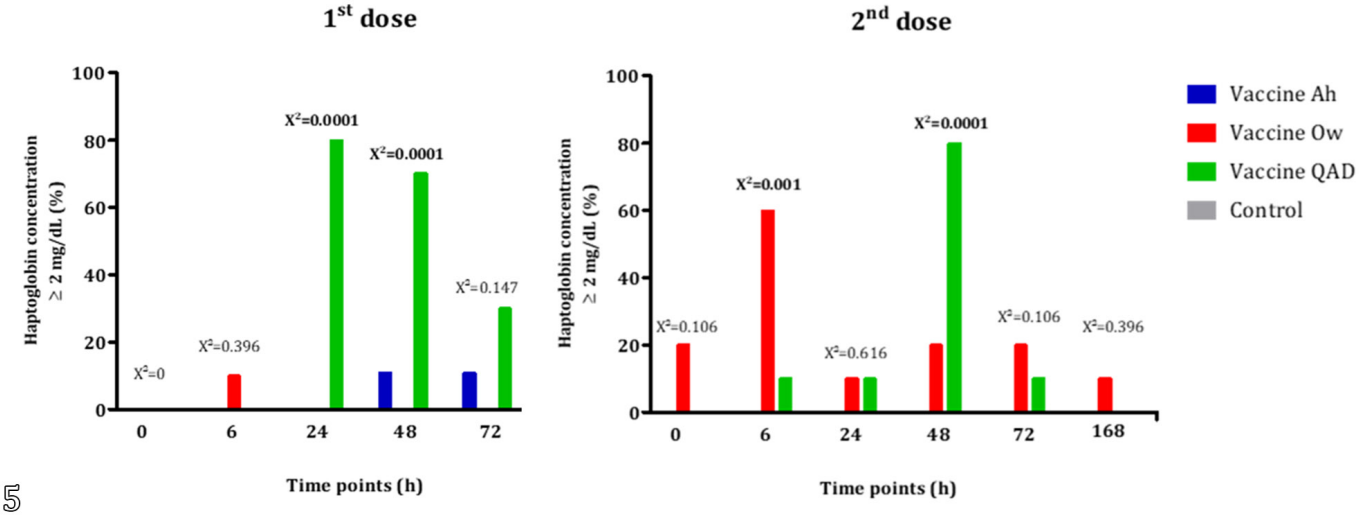

Fig.5. Frequency of heifers comparing Vaccine Ah, Vaccine Ow, Vaccine QAD, and Control group based on haptoglobin concentration (≥2.0mg/dL) after 1st and 2nd doses. X2 = Chi-square test. Differences were considered significant when *P*≤0.05.

involving this type of formulation observed episodes of high haptoglobin concentrations, a significant biomarker for inflammation in bovines. The QAD composition causes local inflammatory reactions that increase the antigen's absorption and extend glycoproteins' stability locally. On the other hand, histologically, a pure fraction of saponin results in tissue toxicity, local granulomas, and intense hemolysis associated with the affinity of saponin to cholesterol (Sjölander et al. 1998). Regardless of the Vaccine QAD composition, vaccinated heifers presented with higher local reactivity levels when considering the increase in temperature at the injection site after the vaccination and second dose compared with non-vaccinated controls.

Vaccines Ow and QAD resulted in higher local inflammatory levels than those induced by Vaccine Ah or the Control group. After being vaccinated, the animals' discomfort was directly related to stress and pain, resulting in decreased food intake, apathy, and milk production. The intense inflammatory reactions could cause cytokine liberation, which acts on the pituitary gland triggering higher aldosterone, cortisol, and catecholamine secretion levels (Chase 2007). Rodrigues et al. (2015) described a high plasma concentration of cortisol and insulin detected in heifers 2 to 16 h after vaccination against bovine respiratory disease. During the inflammatory response, insulin levels increase to amplify the organism's energy utilization to restore homeostasis (Rodrigues et al. 2015).

The rectal temperature of the vaccinated heifers was higher in the experimental groups compared to the Control group in the first 24 h after the first and second doses; however, major peaks were observed in the Vaccine QAD group (Baccili et al. 2019a). However, none of the heifers presented medium (40.1-41°C), high (41.1-42°C), or very high fever (>42°C), according to Feitosa (2014). A few animals from the Vaccine QAD group showed a slight fever from 6 to 24 hours after vaccination. This adjuvant potentially induced intense local inflammation with the exacerbated liberation of proinflammatory cytokines, such as IL-1β, IL-6, and TNF-α, which bond not only to the toll-like receptors of the cells that produce the antigen but also to the adjacent cells. The paracrine effect of proinflammatory cytokines can initiate a more generalized inflammatory response. The circulating proinflammatory cytokines enter the hypothalamus and other brain areas, stimulating prostaglandin E2 production, a substance that induces hyperthermia (Murphy et al. 2008). In the bone marrow, the cytokines act in a way that they induce stroma cells and macrophages to release glycoprotein granulocyte colony-stimulating factor (G-CSF) and the additional production of necessary leukocytes for the development of an immune response (Th1 and Th2) (Catron et al. 2004).

The haptoglobin concentration was presented by Baccili et al. (2019a) with the same experiment, and the findings corroborated with Figure 5, where Vaccine QAD showed a higher frequency of values above 2mg/mL, especially at 24 h after first dose and 48h after second dose. This fact indicates that this adjuvant promotes an acute inflammatory profile faster than other substances. Kim et al. (2021) have reported an increase in acute-phase immune response after the foot-and-mouth disease virus (FMDV) vaccination. These authors described an increase in haptoglobin concentration 8-9 days after the FMDV vaccine was administered. Thus, the present study probably did not find higher haptoglobin values for Vaccine Ow with oil base because the last time point was 168 h after vaccine administration. Due to its composition, new studies are suggested to assess local and systemic adverse effects over a more extended period, particularly for Vaccine Ow. According to these findings, it is evident that the Vaccine QAD formulation induced not only local inflammation that was confirmed by IRT, especially after the second dose, but, also a potential Th1 response that was confirmed by the systemic manifestations observed (rectal temperature and haptoglobin concentration). It results in the activation of an inflammatory cascade owing to the local reaction, cytokine production, and, consequently, the activation of B and T lymphocytes. This fact was explained by Baccili et al. (2019b) with the humoral immune response.

Baccili et al. (2019b) described that Vaccine Ah proved more efficacious in eliciting antibodies against BVDV-1, while it did not induce antibody production against BVDV-2. Nevertheless, Vaccine QAD developed neutralizing antibodies against BVDV-2, and Vaccine Ow exhibited an undetectable reaction against BVDV-1 and BVDV-2. The most effective protective response against BoHV-1 was observed in heifers vaccinated with the modified thermosensitive live vaccine (Vaccine QAD). Therefore, based on the current data regarding adverse reactions measured by IRT and previous studies (Baccili et al. 2019a, 2019b), the Vaccine QAD can be suggested as the best option due to its high antibody production and lower IRT temperature response. However, it should be noted that this adjuvant has shown high systemic reactions (rectal temperature and Hp concentration).

CONCLUSION

Although Vaccine QAD has shown more systemic adverse effects (rectal temperature and haptoglobin concentration) and local effects through cardinal signs of inflammation, Vaccine Ow exhibited higher temperatures as detected by IRT. Hence, this method could be regarded as a tool that contributes to categorizing responses and local inflammation post-vaccination in heifers with reproductive vaccines. In conjunction with assessing systemic manifestations, it allows for monitoring adverse reactions, thereby enhancing the identification of the magnitude of both systemic and local effects.

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