Effect of a phase I *Coxiella burnetii* inactivated vaccine on body temperature and milk yield in dairy cows

L. S.-Ch. Schulze,* S. Borchardt,* V. Ouellet,† and W. Heuwieser*¹

*Clinic for Animal Reproduction, Faculty of Veterinary Medicine, Freie Universität Berlin, Koenigschweg 65, 14163 Berlin, Germany

†Département de Sciences Animales, Université Laval, G1V 0A6 Québec, Canada

ABSTRACT

Q fever is a zoonotic disease caused by *Coxiella burnetii*. The pathogen is prevalent in ruminants (goats, sheep, cows), which are the main sources of human infection. In the cattle industry around the world, animal (15 to 20%) and herd (38 to 72%) level prevalences of *C. burnetii* are high. Vaccination of ruminants against Q fever is considered important to prevent spreading of the disease and risk of infection in humans. However, published information on side effects of the Q fever vaccination under field conditions is limited for cows. The objective of this study was to investigate the effect of the phase I *C. burnetii* inactivated vaccine Coxevac on body temperature and milk yield in dairy cows. In 2 experiments, a total of 508 cows were randomly divided into 2 groups to determine the effect of first vaccination on body temperature and milk yield. The *C. burnetii* serostatus of all cows was tested before vaccination with an indirect ELISA. The first experiment took place in the teaching and research barn of the Clinic of Animal Reproduction at the Freie Universität Berlin. Temperature was measured vaginally in 10 cows in a crossover design. The second experiment was conducted on a commercial dairy farm. Milk yield of 498 cows was measured 1 wk before and 1 wk after vaccination. In a subset of 41 cows, temperature was measured rectally. In both experiments, body temperature increased significantly after vaccination ($1.0 \pm 0.9^\circ\text{C}$ and $0.7 \pm 0.8^\circ\text{C}$). A significant difference was also found in body temperature between vaccinated and control cows. Thirty percent of the vaccinated animals in experiment 1 showed reversible swelling at the injection site as a reaction to the vaccination. The results indicate that

vaccination against Q fever causes a transient increase of body temperature that peaks in the first 12 to 24 h and declines after that. In experiment 2, vaccinated cows (26.8 ± 0.39 kg/d) produced significantly less milk than did control cows (28.2 ± 0.44 kg/d) 7 d after first vaccination. The cumulative milk loss after first vaccination was influenced by an interaction between *C. burnetii* serostatus and average milk yield 7 d before first vaccination. This was considered as part of the physiological immune response. Three out of 10 vaccinated animals in experiment 1 showed painful swelling of the skin at the injection site, which had a maximum size of $14.0 \times 14.0 \times 1.1$ cm. In conclusion, a transient increase of body temperature and a decrease in milk yield is prevalent after Coxevac vaccination.

Key words: *Coxiella burnetii*, Q fever, vaccination, body temperature

INTRODUCTION

Q fever is a zoonotic disease prevalent worldwide that is caused by the gram-negative bacterium *Coxiella burnetii*. *Coxiella burnetii* has the capacity to produce spores that are exceptionally resistant to physico-chemical factors (Bielawska-Drózd et al., 2013), thus surviving well in the environment. It is well known that domestic ruminants are the major reservoirs of *C. burnetii*. Human infections are primarily attributed to sheep and goats (Delsing and Kullberg, 2008) but rarely to cattle (Hellenbrand et al., 2001). In 6 out of 40 Q fever outbreaks in humans between 1944 and 1999 in Germany, the suspected source was cattle (Hellenbrand et al., 2001). The effect of an animal species on the transmission of Q fever to humans presumably depends on the main types of exposure to an animal species in a population, and the infection rate of these animals (Bernard et al., 2012). Risk factors associated with seropositivity include veterinarian procedures such as cattle obstetrics (Bernard et al., 2012), breeding cattle, and any job contact with waste from beef cattle or

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¹Corresponding author: w.heuwieser@fu-berlin.de

goats (Whitney et al., 2013). Cattle are frequently persistently infected, and persistent infection is associated with elevated seroactivity (Guatteo et al., 2007).

In most species, Q fever infection is asymptomatic and can last for a lifetime (Garcia-Ispuerto et al., 2014). In humans, symptoms are usually flu-like, thus often leading to delayed or misdiagnoses and underestimation of cases (Taurel et al., 2014). In more severe cases, Q fever causes abortion, endocarditis, hepatitis, and osteoarticular infection (Parker et al., 2006). In cattle around the world, animal (15 to 20%) and herd (38 to 72%) level prevalence of *C. burnetii* is high (Guatteo et al., 2011). In a German study intensively screening dairy farms in Bavaria, sero- and herd prevalences of *C. burnetii* were $14.8 \pm 0.48\%$ and $72.3 \pm 3.6\%$, respectively (Böttcher et al., 2011). Yet, Q fever symptoms described in the literature have so far been inconsistent (Guatteo et al., 2011). Infertility, abortion (Bildfell et al., 2000), metritis, and mastitis (Arricau-Bouvery and Rodolakis, 2005; Barlow et al., 2008) were commonly reported. The presence of *C. burnetii* in dairy herds has not been clearly demonstrated to negatively affect reproductive performance, and the infection mechanism remains unknown (López-Gatius et al., 2012; Garcia-Ispuerto et al., 2013, 2014).

Recently, the importance of Q fever prevention and control were emphasized by outbreaks in the Netherlands between 2007 and 2010 that infected more than 3,500 humans and led to 7 deaths (van der Hoek et al., 2010). Two main strategies can be used to control and prevent the disease: nonmedical and medical strategies. Nonmedical strategies are mostly hygiene related and focus on the time around parturition as ruminants have been reported to shed large loads of the bacteria at that time (Berri et al., 2002). Their efficiency is poorly documented in the literature (Taurel et al., 2014). Medical strategies include antibiotic therapy and vaccination. Antibiotic therapy is mainly based on the use of tetracyclines. However, the efficacy of tetracycline to reduce shedding is inconsistent (Durand, 1993; Taurel et al., 2012; Taurel et al., 2014), and a blanket treatment is not in accordance with a prudent use of antibiotics. It has been demonstrated, however, that a phase I vaccine is effective to prevent shedding at calving when administered to noninfected animals such as nulliparous animals (Guatteo et al., 2008) and to reduce shedding in infected animals at calving (Arricau-Bouvery et al., 2005). Most recently, it has been reported that a phase I vaccine is effective to reduce the prevalence of animal shedding the bacteria, bacterial load shed in cows (Taurel et al., 2014), and abortion (Arricau-Bouvery et al., 2005). Vaccination also may increase the likelihood of pregnancy by 1.25 (López-Helguera et al., 2013).

Phase I *C. burnetii* inactivated vaccine (Coxevac, Ceva Santé Animale, Libourne, France) against Q fever has been conditionally licensed in the European Union since 2010 and was granted full registration in 2015. Vaccines prepared from phase I *C. burnetii* organisms (virulent phase) are more protective against Q fever in laboratory animals than those prepared from phase II bacteria (Arricau-Bouvery et al., 2005). For goats, swelling at the injection site, increased body temperature, and decreased milk production after vaccination are described by the manufacturer (Ceva, 2012). For cows, only swelling at the injection site was reported by the manufacturer. Although anecdotal evidence is available of fever and decreased milk yield in some dairy cows after vaccination, science-based information is not available. Therefore, the objective of this study was to determine the effect of the vaccine on body temperature, milk yield, and injection site reactions in dairy cows.

MATERIALS AND METHODS

Two experiments were conducted to evaluate the effect of an inactivated phase I vaccine against *C. burnetii* on body temperature and milk yield after first vaccination. In both experiments, a commercial vaccine was used (Coxevac, Ceva Santé Animale, Libourne, France). Each vaccinal dose of 4 mL contained purified corpuscular antigens of phase I *C. burnetii* (100 g/mL) inactivated by formaldehyde. Components of the vaccine are thiomersal, sodium chloride, disodium hydrogen phosphate, potassium dihydrogen phosphate, and water for injections. Coxevac does not contain any adjuvants.

The first experiment was conducted in August and September 2014 at the Clinic of Animal Reproduction, Freie Universität Berlin, Germany (52°25'37"N, 13°14'14"E). A total of 10 clinically healthy Holstein dairy cows were used. They were housed in a freestall barn with cubicles, bedded with a mix of chopped straw and lime. Animals were fed twice daily with grass, silage, concentrate, and hay. Serostatus of all cows was determined using an indirect ELISA (LSIVet Ruminant Q Fever, Life Technologies Corporation, Carlsbad, CA). A serum sample was considered as negative for antibodies against *C. burnetii* when the optical density as a percentage of a positive control (% optical density, OD%) was ≤ 40 . A serum sample was considered as positive for antibodies against *C. burnetii* when OD% was >40 . All cows were negative except one that was questionable.

The cows were randomly divided into 2 groups by use of a random treatment allocation plan generated before

initiation of the trial with the random number function of Excel (version 2013, Microsoft Corp., Redmond, WA). Group I ($n = 5$) was vaccinated and group II ($n = 5$) served as the control. Two weeks later, group II was vaccinated and group I served as the control group. The vaccination was administered subcutaneously with a disposable needle (21 ga $\times$ 1.5) in the neck. Animals were re-vaccinated 21 d after first vaccination. Cows in the control group remained untreated.

Temperature data loggers (DST micro-T, Star:Oddi, Gardabaer, Iceland) were attached to a modified controlled internal drug release device without progesterone (CIDR, InterAg, Hamilton, New Zealand) as previously described and validated (Burfeind et al., 2011; Geiser et al., 2014). The devices were inserted into the vaginal cavity of vaccinated and control cows 1 d before first and second vaccination, respectively. Temperature was measured every 15 min for 92 to 96 h. Afterward, loggers were removed and data were downloaded. For further analyses, hourly means, and means for 6 and 12 h, respectively, were calculated for every cow independently. Vaginal temperatures below 38.0°C were considered as artifacts due to loss or movement of the temperature logger and excluded from further analysis (Burfeind et al., 2011). All cows were clinically examined every morning from 1 d before vaccination until 3 d after vaccination. Examination included inspection of behavior, overall attitude, measurement of rectal temperature, and examination of injection sites. Investigators were blinded to the treatment group.

Ambient temperature (°C) and relative humidity (RH, %) within the barn were recorded hourly using a Tinytag Plus II logger (Gimini Loggers Ltd., Chichester, West Sussex, UK), which was secured at a beam 2.50 m from the ground within the barn. The temperature humidity index (THI) was calculated according to the equation reported by Kendall et al. (2008): $THI = (1.8 \times T + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times T - 26)]$.

The second experiment was conducted in September and October 2014 on a commercial dairy farm in Mecklenburg-Vorpommern, Germany (53°50'44"N, 13°14'40"E) milking a total of 1,200 dairy cows. Four hundred ninety-eight lactating Holstein cows (239 primiparous, 259 multiparous) were included. The cows were housed in 2 freestall barns with cubicles bedded with straw and fed a TMR 2 times a day. Cows were milked twice daily in a 50-stall rotary milking parlor (DeLaval, Tumba, Sweden). Individual milk yield was recorded for each milking from 7 d before vaccination (d -7) through 7 d after vaccination (d +7). Milk production data were captured using an on-farm computerized application that recorded milk production and

herd management information (Herde, dsp-Agrosoft, Paretz, Germany).

The cows were randomly divided into 2 groups using the European cow registration number, whereby even-numbered cows were vaccinated and odd-numbered cows were kept as controls. Group I ($n = 246$) was vaccinated and group II ($n = 252$) served as an untreated control. Vaccination, dosage, and application were identical as described in experiment 1. Animals were re-vaccinated 21 d after first vaccination. Serostatus was assessed as described in experiment 1. One hundred seventy-five cows were positive (35%), and 323 cows were negative (65%).

Data were statistically analyzed with SPSS (version 22, IBM, Ehningen, Germany). Differences in vaginal (experiment 1) or rectal temperature (experiment 2) before and after vaccination were determined with a paired *t*-test comparing the corresponding values in each group. An independent samples *t*-test was used to investigate the differences in temperature between vaccination and control groups. To study the effect of vaccination on milk yield, a repeated measure analysis was performed in a GLM framework using SPSS. To test whether the milk production response to the treatment depended on the initial level of milk production (7 d before vaccination) of the subjects, 3 groups were built based on the average milk yield 7 d before vaccination (group A: 9.0–25.2 kg/d; group B: 25.3–30.6 kg/d; group C: 30.7–47.6 kg/d). Cows with more than 4 missing milk recordings were excluded from the analysis ($n = 265$). To determine the effect of vaccination on milk production, we evaluated the daily milk yield over the study period (i.e., d -7 until d +7) and the cumulative milk loss (δMY). Therefore, δMY was calculated by subtracting the cumulative 7 d milk yield after vaccination (i.e., d +1 until d +7) from the cumulative milk yield before vaccination (i.e., d -1 until d -7). Subsequent analysis of the effect of vaccination on δMY was performed by GLM. Fixed effects were vaccination (yes, no), initial level of milk production (group A, group B, group C), *C. burnetii* serostatus before vaccination (positive/negative), and lactation group (primiparous; multiparous). Additionally a 3-way interaction was built with vaccination, initial level of milk production, and *C. burnetii* serostatus before vaccination. Initially, we also considered pregnancy status (pregnant vs. nonpregnant) at vaccination in the model. However, when tested in an univariate model as a fixed factor, no association was found with δMY ($P = 0.611$). Therefore, we excluded this factor from the final analysis as described previously (Dohoo et al., 2009).

Table 1. Body temperature (°C; mean $\pm$ SD, 75th percentile) of vaccinated and not vaccinated cows before (baseline) and after vaccination

Time relative to vaccination	Vaccinated		Not vaccinated	
	Mean $\pm$ SD	75th percentile	Mean $\pm$ SD	75th percentile
Experiment 1		n = 10		n = 8
0–12 h before (baseline)	38.4 $\pm$ 0.4 ^a	38.5	38.4 $\pm$ 0.1 ^a	38.5
12–24 h after vaccination	39.4 $\pm$ 0.8 ^b	40.2	38.3 $\pm$ 0.2 ^a	38.5
Experiment 2		n = 21		n = 19
Before vaccination	38.5 $\pm$ 0.2 ^c	38.6	38.5 $\pm$ 0.3 ^c	38.6
Day after vaccination	39.1 $\pm$ 0.8 ^d	39.5	38.5 $\pm$ 0.3 ^c	38.8

^{a–d}Means within a row with different superscripts differ ($P < 0.05$).

Rectal temperature was measured in a subset of 41 cows (24 primiparous, 17 multiparous) immediately before vaccination and 24, 32, and 48 h after vaccination with a digital thermometer (Microlife VT 1831, Microlife AG, Widnau, Switzerland) as previously described (Burfeind et al., 2010). Fourteen cows were seropositive (34%) and 27 cows seronegative (66%). Ambient temperature and RH were recorded accordingly to experiment 1. The THI was calculated as described above.

RESULTS

Daily ambient temperatures (mean $\pm$ SD) were 19.3 $\pm$ 2.3°C and 17.7 $\pm$ 2.7°C in experiments 1 and 2, respectively. Daily THI were 65.3 $\pm$ 3.3 and 63.3 $\pm$ 4.3, respectively.

In experiment 1 (Table 1), basal vaginal temperatures of cows in group I and group II were similar ($P = 0.599$). Mean vaginal temperature of the vaccinated cows increased by 1.0°C within 12 to 24 h after treatment ($P = 0.005$). Seven out of 10 (70%) vaccinated animals were febrile (temperature $\geq 39.5^\circ\text{C}$) 11.1 $\pm$ 2.6 h (mean $\pm$ SD) after the vaccination. The vaginal temperature of febrile cows remained elevated for 3.0 to 46.8 h and reached its maximum of 41.3°C 4.3 $\pm$ 4.2 h (mean $\pm$ SD) after the vaccination. The difference of mean vaginal temperatures between vaccinated and control cows was 0.4°C ($P = 0.009$), 1.1°C ($P = 0.002$), and 0.4°C ($P = 0.029$) 0 to 12 h, 12 to 24 h, and 24 to 36 h after vaccination, respectively. Within the control

group, the mean vaginal temperature did not differ between the times of measurements. One control cow had to be excluded from temperature analysis because of the development of fever (body temperature $\geq 39.5^\circ\text{C}$) before time of vaccination. Three of the vaccinated cows showed a painful swelling of the skin at the injection site that had a maximum size of 14.0 $\times$ 14.0 $\times$ 1.1 cm. The swelling appeared in 2 cows at the first day after vaccination and in 1 cow at the third day after vaccination. Swellings disappeared within a few days.

In experiment 2 (Table 1), basal rectal temperature of cows (i.e., before vaccination) in both groups was also similar ($P = 0.172$). Mean rectal temperature of vaccinated cows increased by 0.7°C ($P = 0.001$) after vaccination. Ten out of 21 (48%) vaccinated animals were febrile. The maximum body temperature of vaccinated cows was 41.8°C. No difference was observed in mean rectal temperature in the control group between basal values and the day after vaccination. The mean rectal temperature of vaccinated cows was 0.6°C higher ($P = 0.003$) than the mean rectal temperature of control animals on the first day after vaccination and 0.3°C higher on the second day after vaccination ($P = 0.018$), respectively. One cow in the control group had to be excluded from temperature analysis because of the development of fever (body temperature $\geq 39.5^\circ\text{C}$) before time of vaccination.

Milk production (kg/d) of cows in both groups was similar (not vaccinated, 28.4 $\pm$ 0.42; vaccinated, 28.2 $\pm$ 0.42; $P = 0.826$) before vaccination (Table 2). Vac-

Table 2. Descriptive statistics of Holstein cows enrolled in experiment 2

Item	Vaccinated (n = 252)	Not vaccinated (n = 246)
Primiparous cows, no. (%)	115 (45.6)	124 (50.5)
Multiparous cows, no. (%)	137 (54.4)	122 (49.5)
DIM at vaccination ($\pm$ SEM)	164.2 (± 6.10)	165.7 (± 6.07)
Average daily milk yield before vaccination, kg/d ($\pm$ SEM)	28.2 (± 0.41)	28.4 (± 0.43)
Average daily milk yield after vaccination, kg/d ($\pm$ SEM)	26.8 (± 0.39) ^a	28.2 (± 0.44) ^b
Seronegative, no. (%)	156 (61.9)	167 (67.9)
Seropositive, no. (%)	96 (38.1)	79 (32.1)

^{a,b}Means within a row with different superscripts differ ($P < 0.05$).

Table 3. Effect of a *Coxiella burnetii* vaccination and serostatus before vaccination on cumulative milk loss (means $\pm$ SEM) considering pretreatment milk yield (means $\pm$ SEM)

Group	Serostatus	Average daily milk yield before vaccination, kg/d	No.	Cumulative milk loss 7 d after vaccination, kg
Control	Negative	21.1 $\pm$ 0.37	59	1.2 $\pm$ 2.34
		27.8 $\pm$ 0.23	51	-4.1 $\pm$ 2.50
		36.3 $\pm$ 0.53	57	-2.1 $\pm$ 2.39
	Positive	22.8 $\pm$ 0.51	24	-1.0 $\pm$ 3.68
		27.9 $\pm$ 0.27	33	-3.9 $\pm$ 3.14
		36.0 $\pm$ 1.03	22	-1.1 $\pm$ 3.84
Treatment	Negative	21.6 $\pm$ 0.50	44	-8.2 $\pm$ 3.00
		28.1 $\pm$ 0.20	49	-11.8 $\pm$ 2.86
		35.4 $\pm$ 0.46	62	-17.7 $\pm$ 2.54
	Positive	20.5 $\pm$ 0.63	39	0.0 $\pm$ 3.20
		27.7 $\pm$ 0.27	32	-5.2 $\pm$ 3.54
		34.7 $\pm$ 0.67	25	-8.8 $\pm$ 4.00

inated cows produced less milk after vaccination (not vaccinated, 28.2 ± 0.44 ; vaccinated, 26.8 ± 0.39 ; $P = 0.033$). The cumulative milk loss after vaccination was significantly influenced by vaccination ($P = 0.001$) and lactation group ($P = 0.001$). A tendency was found for the 3-way interaction of vaccination, serostatus, and milk production group ($P = 0.086$). Seronegative cows with the highest milk production had the highest cumulative milk loss (Table 3; Figures 1 to 4).

DISCUSSION

Convincing evidence is available that body temperature is a useful and sensitive parameter to study the reactions of animals to physiological functions (e.g., nutrition, lactation, and reproduction), environmental challenges, and disease processes (Nakamura and Shimizu, 1983). An increase of body temperature as a side effect of vaccinations against bacterial and viral

Figure 1. Mean daily milk yields ($\pm$ SEM; $n = 498$) in vaccinated (■) and nonvaccinated (○) Holstein cows 7 d before to 7 d after first vaccination with an inactivated *Coxiella burnetii* vaccine.

Figure 2. Effect of an inactivated *Coxiella burnetii* vaccine on cumulative milk loss (mean $\pm$ SEM) 7 d after first vaccination ($n = 498$) considering 3 pretreatment milk production levels. (A) 9.0 to 25.2 kg/d, open box; (B) 25.3 to 30.6 kg/d, shaded box; (C) 30.7 to 47.6 kg/d, black box.

Figure 3. Effect of *Coxiella burnetii* serostatus on cumulative milk loss (mean $\pm$ SEM) 7 d after first vaccination in vaccinated animals ($n = 252$) considering 3 pretreatment milk production levels. (A) 9.0 to 25.2 kg/d, open box; (B) 25.3 to 30.6 kg/d, shaded box; (C) 30.7 to 47.6 kg/d, black box.

pathogens has been documented. After administration of two 9-way vaccines to 164 cows, a significant increase ($P = 0.001$) in mean temperature of vaccinated animals between d 0 and 1 occurred (Scott et al., 2001). Mean temperature of vaccinated cows was significantly higher on d 1 compared with control cows (vaccine 1: 0.41°C ; vaccine 2: 0.29°C). Also, an inactivated bovine herpesvirus 1 vaccine tested in 455 lactating cows in the Netherlands caused a significant increase (0.45°C) in body temperature (Bosch et al., 1997). In both studies, reporting of the temperature measurement was unclear. Whereas in the first study temperature was measured electronically once a day from 2 d before until 10 d after vaccination (Bosch et al., 1997), in the second study no details were provided at all except that measurements were rectal (Scott et al., 2001). To closely study relationships between changes in temperature and vaccination, we used devices logging the vaginal temperature every 15 min from 1 d before to 92 to 96 h after vaccination in experiment 1. In both studies, body temperature was measured with rectal thermometry 3 times in 2 d in experiment 1 and immediately before vaccination and 24, 32, and 48 h after vaccination in experiment 2, respectively. We found a significantly elevated body temperature after vaccination in both

experiments of the present study. Seventy and 48 percent of vaccinated cows developed fever in experiments 1 and 2, respectively.

Body temperature is influenced by heat stress during different stages of lactation (Hahn, 1999; Kadzere et al., 2002; Mader et al., 2006). A THI of 72 equivalent to 25°C ambient temperature and 50% relative humidity is generally accepted as the upper threshold of the comfort zone for cattle (Igono et al., 1992; West et al., 2003; Kendall et al., 2006). In the present study, average daily THI were 65.3 ± 3.3 and 63.3 ± 4.3 (mean $\pm$ SD), respectively. Therefore, we can exclude an influence of THI on body temperature in our data.

The lower proportion of febrile cows and the smaller difference in temperature between vaccinated and control animals in experiment 2 compared with experiment 1 are likely due to the different measurement methods. Whereas in experiment 1, body temperature was measured in the vagina every 15 min, in experiment 2 body temperature was measured in the rectum 3 times in 2 d. Thus, the experiments had differences in frequency and site of measurement, and measurement device, respectively. It is obvious, that through high frequency measurements the temperature profile will be detected more accurately and the risk to miss peak temperatures

Figure 4. Effect of *Coxiella burnetii* serostatus on cumulative milk loss (mean $\pm$ SEM) 7 d after first vaccination in nonvaccinated animals (n = 246) considering 3 pretreatment milk production levels. (A) 9.0 to 25.2 kg/d, open box; (B) 25.3 to 30.6 kg/d, shaded box; (C) 30.7 to 47.6 kg/d, black box.

is reduced. Scott et al. (1998) suggested that a 24-h sampling interval from treatment administration until first post treatment measurement could be too long to detect pyrexia changes. In the present study, cows developed fever 11.1 ± 2.6 h (mean $\pm$ SD) after vaccination. The temperature of febrile cows remained elevated for 3.0 to 46.8 h. With a measurement frequency of once a day, only 3 out of 7 febrile cows would have been detected by rectal measurement. Regarding the device of measurement, it has been shown that rectal measures can be biased by intra-observer variability, penetration depth, and thermometer type (Burfeind et al., 2010). Temperature data of vaginal loggers can be influenced by logger movement resulting in the sensor end pointing to the external environment. According to Burfeind et al. (2011), we considered vaginal temperatures below 38.0°C as artifacts due to loss or movement of the temperature logger and excluded those from further analysis. Thus, we do not expect an influence of logger movement or loss on our results. We assume that both measurement frequency and measurement method can explain the differences in temperature data between the 2 experiments. We do not assume that the different sites of measurement had an effect because the association between rectal and vaginal temperature using identical loggers is high and the difference negligible (Suthar et al., 2013).

Milk production can be also considered as a physiological function of lactating dairy cattle sensitive to stressors. Therefore, we evaluated the effect of vaccination on short-term changes in milk production. The cumulative milk loss after first vaccination was influenced by an interaction between *C. burnetii* serostatus and average milk yield 7 d before first vaccination. High-producing cows testing negative for *C. burnetii* before first vaccination had the highest cumulative milk loss 1 wk after vaccination. Although several studies report a negative effect of vaccination on short-term milk production for different kind of antigens (Musser and Anderson, 1996; Scott et al., 2001; Bergeron and Elsener, 2008), this is the first study showing an effect of a phase I *C. burnetii* inactivated vaccine. It was also reported before that milk loss in high-producing cows is more pronounced when a 9-way killed vaccine was used (Scott et al., 2001). To our knowledge, this is the first study showing an effect of *C. burnetii* serostatus before vaccination on the physiological response in vaccinated lactating dairy cows. The physiological response in an animal naive to the inactivated phase I antigen of *C. burnetii* seems to be more pronounced compared with a seropositive animal.

In both experiments of this study, control animals remained untreated. Several vaccination trials included

a placebo-treated control group. Some inactivated vaccines contain adjuvants that are well known to cause side effects (Gethmann et al., 2009). In those cases, it makes sense to include placebo-treated animals that receive an injection of adjuvant containing the carrier or saline to compare whether the vaccine antigens or the adjuvant causes the effects. Coxevac did not contain any adjuvant. Therefore, we refrained from a placebo treating of the control cows. The principal difference between a no-treatment controlled trial and a placebo-controlled trial is that subjects and investigators are not blind to treatment assignment (Department of Health and Human Services, 1999). Vaccinated and untreated cows were restrained for the same time and thus the disruption of their feeding and social routines were identical. These actions should equally apply to the control and treatment group, even if it is not strictly necessary to deliver an untreated status.

Swelling of the injection site is an adverse event that is observed frequently after vaccination of cattle with different vaccines. Gethmann et al. (2009) investigated the safety of 3 different monovalent vaccines against blue tongue virus in 1,007 sheep and 893 cattle. The blue tongue virus vaccines were administered subcutaneously at the neck like the Coxiella vaccine in the present study. Short-term moderate swelling of the injection site was seen after the first vaccination only in 2 juvenile cows, but more frequently in adult cows. After the second vaccination, virtually all cows reacted with low-grade swelling. Significant differences in swelling at the injection site between vaccinated and control animals was also reported in a study investigating the effect of a core antigen vaccine against gram-negative bacteria on physiologic and yield parameters of dairy cows (Scott et al., 1998).

Aside from differences in frequency of febrile cows and temperature differences between vaccinated and control animals, we found a moderate but significant, transient increase of body temperature in both experiments, temporary swelling of the injection site in experiment 1, and a reduction in milk yield in experiment 2. Rising temperatures after vaccination can be interpreted as a physiological systemic reaction, which is common after the application of many vaccines (Gethmann et al., 2009). In conclusion, a transient increase of body temperature and decrease in milk yield is prevalent after Coxevac vaccination as part of the physiological immune response.

According to the manufacturer's specification, initial immunization for *C. burnetii* with Coxevac should be completed 3 wk before first artificial insemination in heifers. Results from our study indicate that cows tested positive for *C. burnetii* did not show a pronounced side

effect on milk yield due to the initial immunization. Although not tested specifically, one can conclude that seroconversion in heifers due to primary vaccination might protect them from a marked physiological immune response with negative effects on milk production when these animals are revaccinated during lactation. Therefore, to avoid negative effects of vaccination on milk production, we recommend initial immunization of heifers in agreement with the manufacturer's specification. This should be evaluated in further studies.

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