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Investigation of humoral and cellular immunity of dairy cattle after one or two year of vaccination with a phase I *Coxiella* vaccine

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Abstract

Q fever is a worldwide zoonosis that may cause reproductive disorders such as abortion, endometritis or infertility in livestock. The implementation of a vaccination with a phase I vaccine is the nowadays the most relevant way to control the spread of the infection within herds. Annual boosters are recommended for ruminants by the manufacturer whereas in humans, to prevent side effects, no booster must be done before 5 years and the lack of humoral and cellular immunity has to be confirmed before any additional vaccination. The aim of this study was to investigate, in dairy cattle, the interest of such annual booster by assessing the level of different immune markers among 142 animals (from infected and uninfected herds) vaccinated either 2 year (*i.e.* 2 times) or 1 year before with an efficient commercial phase I Q fever vaccine. One year after vaccination, more than 80 % of the vaccinated cows had still immune markers, whereas 68 % of the heifers from uninfected herd did not. These data suggested that an annual booster would not be necessary for all vaccinated animals within a herd. In order to detect the immune animals and then to optimize the number of animals needing a boost, the skin test method, performed at least 3 days before the vaccination could be used.

Keywords: Coxiella burnetti; vaccine; dairy cattle; humoral immunity; cellular immunity.

1. Introduction

Coxiella burnetii, the causative agent of Q fever, a zoonosis of worldwide distribution, infects various animal species. *C burnetii* is associated with pneumonia and reproductive disorders in livestock including abortion, stillbirth, delivery of weak and unviable newborns, endometritis and infertility [1]. Ruminants are considered as the most common reservoir for human infections as infected ruminants shed high load of *C burnetii* in placenta and parturition products but also in feces and urine, which are the source of environmental contaminations causing

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human diseases. *Coxiella burnetii* may also be shed via milk but oral transmission of the bacteria is considered as less common as well as the possibility of vertical and sexual transmission [1]. Human Q fever usually manifests as a flu-like, self-limiting acute illness although some infections develop into a severe and sometimes fatal chronic disease [2]. Within dairy herd, the implementation of vaccination is nowadays the most effective control measure to prevent and control *C. burnetii* shedding. C *burnetii* undergoes a phase variation in which smooth-LPS virulent phase I converts to rough-LPS avirulent phase II upon serial passage in non immunocompetent cells. Only phase I vaccines are effective in reducing both abortion and shedding of the bacteria in milk, vaginal mucus and feces of ruminants [3,4].

In veterinary medicine, annual boosters are often stipulated for inactivated vaccines, but their grounds are rarely studied. For Q fever, annual boosters are recommended for ruminants by the manufacturer whereas in humans, to prevent side effects, no booster must be done before 5 years and the lack of humoral and cellular immunity has to be confirmed using antibody level determination and skin test before any additional vaccination [5]. Therefore, the aim of this study was to investigate, in dairy cattle, the level of different immune markers among 142 animals vaccinated 2 year (i.e. 2 times) or 1 year (i.e. one time) before with a commercial phase I Q fever vaccine (Coxevac® CEVA Santé Animale Libourne France) in order to confirm or not the interest of an annual booster with such a vaccine.

2. Materials and Methods

To reach this goal, forty-four animals (22 heifers and 22 cows) from an uninfected herd and 98 animals (42 heifers and 56 cows) from 5 infected herds with abortions due to Q fever (confirmed by positive PCR on placenta) were included in the study. The uninfected herd showed a within herd seroprevalence of 0%. As the immune response could be influenced by naturally acquired immunity, only cows tested both negative in ELISA and in PCR (on concomitant samplings of vaginal mucus and milk) before vaccination, and still PCR negative-tested one year after last injection were included in the study [4]. Among these 142 animals, 36 animals from infected herds have been vaccinated the 2 preceding years, i.e. have received a primo-vaccination and a annual boost (VAC 2) and 106 have been vaccinated for the first time the preceding year (VAC 1).

To investigate the cellular immune response, we used a skin test (ST) method consisting in an intradermal inoculation of 0.1 mL of the vaccine diluted 1/3 in the neck followed by a reading 72 to 96 h after. The ST was considered as positive when a nodular area > 1cm was observed.

To investigate the humoral immune response the level of antibody in serum samples was determined by ELISA using Ruminant serum Q Fever LSI kit (LSI, Lissieu, France), the day of the skin test (day 0) and 10 days after. The results were expressed in optical density Sample/Positive control (S/P) ratio. A serum was considered positive when S/P ratio > 40. To assess the booster effect of the skin test an Index S was calculated from the comparison of the antibody titer on day 0 (ATD0) and the antibody titer on day 10 (ATD10). S was estimated as follows: (ATD10-ATD0)/ATD0. When both ATD0 and ATD10 were <40 then S = 0; and when ATD0=0 and ATD10 > 40, then S = 2. We considered than the skin test induced a booster effect if we observed an index S > 0.3 according to the data of unvaccinated control animals in uninfected herd (data not shown).

To investigate the putative differences according to the vaccination scheme (VAC1 *vs* VAC2), the lactation stage of animals (cows *vs* heifers) or the status of the herd (infected *vs* uninfected), we compared distribution among different groups using χ^2 test for qualitative values and Kruskall-Wallis test for the quantitative values. In a first step, we compared the number of seropositive animals at day 0 according to their vaccination scheme and their status at first vaccination. In a second step, the distribution of skin-tested positive or negative animals were compared according to their vaccination scheme and their status at first vaccination. Lastly, the putative booster effect was investigated when comparing the index values according to the vaccination scheme.

3. Results and discussion

One year after the last injection of vaccine, 125 out of 142 animals were still ELISA positive. The number of positive animals (heifers and cows together) and the mean antibody titer were significantly higher ($p < 0.006$ and $p < 0.02$ respectively) in the animals vaccinated the 2 preceding years *(i.e.* 2 times, VAC2) than in the animals vaccinated for the first time the year before *(i.e.* 1 time, VAC1) (mean antibody titer = 109 ± 66 and 75 ± 84,

respectively). However, among the subsample of cows, the number of positive animals was not significantly different between VAC1 and VAC2 groups. The difference observed in the number of positive animals was due to the age at the time of vaccination, more than to the number of vaccinations. Indeed, while 16 heifers out of 21 vaccinated before 14 months of age were still seronegative one year after vaccination, only 4 out of 16 heifers vaccinated after 18 months of age were still seronegative after vaccination. Among heifers first vaccinated after 18 months of age, the proportion of seropositive animals one year after the last injection of vaccine was not different according to the number of vaccination (VAC1 or VAC2) (Table 1).

Table 1 Distribution of seropositive and seronegative animals on day 0 according to their lactation number (heifers vs cows), their age at first vaccination, their vaccination scheme (VAC1 *vs* VAC2) and their belonging herd.

ELISA on the day of skin test	Infected herds					Uninfected herd	
	Heifers age when 1st vaccinated			Cows			
	<14 months	>18 months		VAC1	VAC2	Heifers	Cows
	VAC1[a]	VAC1	VAC2				
Positive[b]	5 (24%)	12 (75%)	4 (80%)	18 (72%)	26 (84%)	3 (14%)	15 (70)
Negative	16 (76%)	4 (25%)	1 (20%)	7 (28%)	5 (16%)	19 (86%)	7 (30)
Total	21	16	5	25	31	22	22

a) VAC1 = animals vaccinated for the first time 1 year before, VAC2 = animals vaccinated the 2 preceding years (i.e. 2 times)
b) Positive = optical density Sample/Positive control (S/P ratio) ≥ 40

Regarding cellular immunity, 91 animals out of 142 (64%) were considered as skin-tested positive (Figure 1)

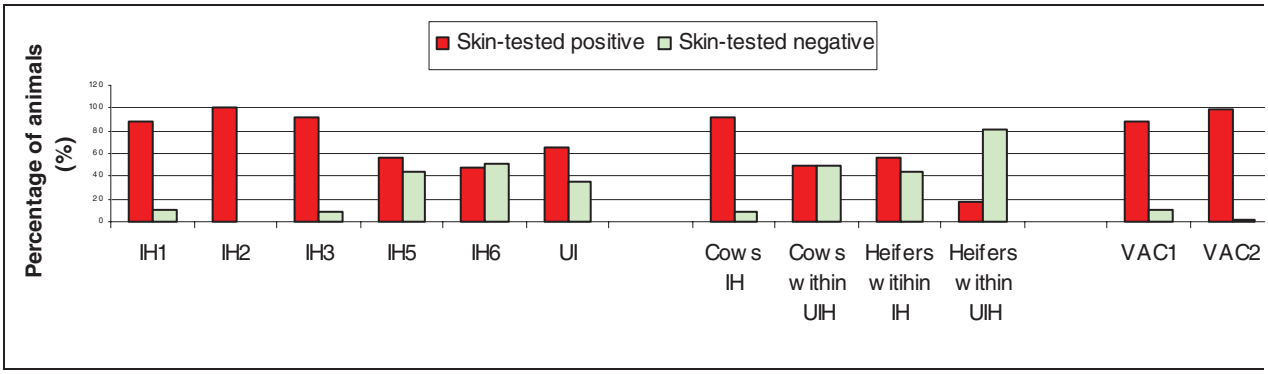

Figure 1. Distribution of skin-tested positive and negative animals according to their herd, their lactation status (cows *vs* heifers) and their vaccination scheme (IH: infected herd)

As for the humoral response, there were significantly less skin-tested positive animals among heifers than in cows ($p < 0.001$) regardless the type of comparison (Fig 1), whether all the cows were compared to all the heifers, ($p < 0.01$) or only the cows and the heifers vaccinated for the first time the preceding year (VAC1 group), within infected ($p < 0.05$) or in the uninfected herd ($p < 0.05$).

The number of vaccination (VAC1 or VAC2) did not significantly increase the number of skin-tested positive cows (Figure 1). However, the number of skin tested positive animals was significantly higher in the infected herds (IH) than in the uninfected one (UIH) regardless the type of comparison: whether all the animals were compared ($p < 0.001$) or only the animals vaccinated one year before (VAC1) ($p < 0.001$), or when the cows or the heifers vaccinated for the first time the preceding year in the infected herds were respectively compared to the cows or the heifers vaccinated for the first time the preceding year in the uninfected herd ($p < 0.01$ in both cases). This difference of response could be due to the presence of some *Coxiella burnetii* in the environment and then bacterium spread between cows within the infected herds which boosted the immunity of the vaccinated cattle contrarily to the animals located in the non infected herds were no antigenic stimulation was provided.

To determine a putative booster effect of the skin-test on the humoral response, the antibody titers before and after the skin test (10 days after) were compared. The antibody titers were significantly higher ($p < 0.02$) 10 days

after the skin-test (mean titer 111 ± 81) than the day of the skin-test (mean titer 87 ± 79) highlighting that the small amount of antigen injected for the skin-test was sufficient to boost the production of antibodies.

The number of animals experiencing a boosted antibody response (65/139), as defined by an index $S \geq 0.3$, did not differ significantly either between skin-tested positive or negative animal, or between seropositive or seronegative animals (Table 2). The number of vaccination and the age at the time of vaccination did not significantly affect the number of animals presenting a booster effect. However there were significantly less heifers presenting a booster effect in the uninfected herd than in the infected herds ($p < 0.05$).

Table 2. Distribution of animals experiencing or not a booster effect according to their skin-test and serological statuses and their lactation number

Booster effect	Skin test		ELISA		Infected herds				Uninfected herd	
					Heifers		Cows		Heifers	Cows
	Neg	Pos[a]	Neg[b]	Pos[b]	Vac1[c]	Vac2[c]	Vac1[c]	Vac2[cd]		
Yes	23	40	30	33	21	3	13	9	6	11
No	28	50	32	46	16	2	10	23	16	11

a) Pos = the skin test positive was considered positive when a nodular area > 1cm was observed.

b) Neg = ELISA negative S/P ratio < 40; Pos S/P ratio ≥ 40

c) VAC1 = animals vaccinated for the first time 1 year before (*i*.e. 1 time), VAC2 = animals vaccinated the 2 preceding years

d) one blood sample was missing, excluding one cow for study of the booster effect.

The use of skin-test followed by the detection of subsequent antibody response was supposed to be a relevant method to assess the presence of a post-vaccination immunity [6] because it could be a sign of the ability of the animals to react to the contact of virulent *Coxiella burnetii*. The present results seemed indicate that the heifers from the uninfected herd were less protected (in terms of duration of protection) than the animals located in infected herds included in this study. Indeed one year after vaccination, 84 % (119/142) of the animals still exhibited a humoral and/or cellular immune response or a booster effect. But among the 24 animals without any immune response 17 (70.8%) were from the uninfected herd and 15 of which were heifers. Sixty eight percent of the heifers in the uninfected herd were skin-tested negative and ELISA-tested negative and did not become ELISA positive after the skin test. In contrast, only 5/42 (11.9 %) heifers and 2/56 (3.6 %) of the cows located in infected herds and 3/22 (11.6 %) of the cows of uninfected were detected negative in every test.

4. Conclusion

This study suggested that while a very low proportion of cows vaccinated one time in infected herds need a booster during the second year, this annual booster seemed very relevant for heifers especially in uninfected herds. The skin test performed at least 3 days before vaccination could be a useful tool to detect the animals with a sufficient cellular immunity and then to optimize the selection of target animals to revaccinate. In routine practice, due to the quite systematic susceptible status of heifers, the vaccination could be implemented as soon as possible (at 3-4 months of age for example) with an annual booster before the first artificial insemination, leading to both an expected maximum booster effect (present study results) and a prevention effect of *C. burnetii* shedding [4].

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