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FAILURE OF Q FEVER PHASE I CORPUSCULAR VACCINE TO INFLUENCE THE PERSISTENCE AND REACTIVATION OF *COXIELLA BURNETII* INFECTION IN MOUSE AND GUINEA PIG TISSUES

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Summary. — In mice and guinea pigs infected with *Coxiella burnetii*, accumulation of large numbers of rickettsiae in the spleen and liver occuring at the early stages of infection was followed by clearing of these organs from the infectious agent but by its persistence in kidneys and reproductive tract at later intervals. The persistence of *C. burnetii* was not affected when one to six months-infected mice or guinea pigs were immunized with 100 or 500 μg of Q fever phase I corpuscular vaccine. Administration of the vaccine also did not substantially influence the reactivation of *C. burnetii* infection elicited in mice by parturition or developing in mice and guinea pigs upon treatment with cortisone or cyclophosphamide (CPA). Some differences observed between groups of immunized or non-immunized animals were only quantitative in nature. The possibility to disbalance the steady state of persisting *C. burnetii* in the host tissues by inhibition or stimulation of the immune response is discussed.

Key words: *Coxiella burnetii*; *persistence*; *laboratory animals*; *Q fever vaccine*; *immunosuppression*; *reactivation*

Introduction

In the series of field studies (Sádecký *et al.*, 1974; Sádecký *et al.*, 1975; Sádecký and Brezina, 1977; Sádecký, 1978; Sádecký and Ábel, 1978), vaccination of cattle and ewes with Q fever phase I corpuscular vaccine prevented shedding of *C. burnetii* in milk and placenta, respectively. Because the shedding of *C. burnetii* was influenced by Q fever phase I corpuscular vaccine also in groups of naturally infected dairy cows and ewes, question arose whether vaccination could influence the persistence of *C. burnetii* in animal tissues in terms of total clearance of the infectious agent. For this reason the presence of *C. burnetii* was followed in tissues of mice and guinea pigs which were immunized with Q fever phase corpuscular vaccine one

to six months after *C. burnetii* infection. Experiments were included on cortisone- or CPA-treated mice and guinea pigs, and on mice which became pregnant during these investigations.

Materials and Methods

C. burnetii strain Nine Mile in the phase I (the 3rd chick embryo yolk sac passage) was partially purified and concentrated by differential centrifugation. Rickettsial suspension pooled in brain-heart infusion (BHI) and stored at −30 °C containing $10^{8.1}$/ml EID_{50} (egg infectious doses) was used for infection of animals. Formalin-killed purified cells of the same strain (Q fever phase I corpuscular vaccine) were used for immunization. They were adjusted to concentration 2 mg/ml in phosphate buffered saline (PBS) and stored at −30 °C. Required concentrations (500 μg for guinea pigs and 100 μg for mice) in 0.5 ml amounts were prepared by diluting the vaccine in PBS.

Experimental animals were adult guinea pigs and SPF mice from breed VELAZ. Approximately equal numbers of each sex were employed. Animals were housed in cages containing 2−3 guinea pigs or 4−6 mice. Prior to use about 20% of guinea pigs and mice were bled from the heart and from the orbital sinus, respectively; sera were tested for the presence of antibodies to *C. burnetii* by the microagglutination (MA) test.

Guinea pigs were infected intraperitoneally (i.p.) with 10^4, mice i.p. with 10^6 EID_{50} of *C. burnetii*. One to six months post infection (p.i.) one half of animals was immunized with Q fever phase I corpuscular vaccine. Ten days later, both immunized and non-immunized animals were divided into 3 groups; each group contained animals not differing more in their weights than 10% as well as not differing significantly in the phase I and phase II MA antibody response. One group was treated with CPA (Rudolstadt, G.D.R.), one group with hydrocortisone (SPOFA, Prague, Czechoslovakia), one group remained untreated. Altogether 6 groups of animals were formed (each containing 3−4 guinea pigs or 4−6 mice) designated as follows: control (non-immunized, untreated), Im-control (immunized, untreated), cortisone (non-immunized, cortisone-treated), Im-cortisone (immunized, cortisone-treated), CPA (non-immunized, CPA-treated), and Im-CPA (immunized, CPA-treated). In addition, two groups of non-immunized and immunized animals were formed designated as pregnant and Im-pregnant.

CPA- or cortisone-treated animals were injected subcutaneously (s.c.) with 7 consecutive doses for 7 days of the drugs in 0.3 ml amounts per mice and in 0.6 ml amounts per guinea pigs. One dose of CPA contained 100 mg/kg body weight of the drug per animal per day; cortisone was given in 1.5 mg doses per mouse per day and in 15 mg doses per guinea pig per day. One day after the end of drugs' treatment, i.e. 18 days after administration of Q fever phase I corpuscular vaccine, animals were killed in chloroform anaesthesia and their blood, spleen, kidneys and reproductive organs (testes or uterus and ovaries) were aseptically removed and further tested.

Determination of tissue infection with C. burnetii. Impression smears prepared were examined microscopically for the presence of *C. burnetii* after staining by Gimenez method and by immunofluorescence (IF) technique. Both procedures were also used for demonstration of *C. burnetii* in other organs (liver, lung, brain, retroabdominal lymph nodes and peritoneum) after i.p. infection. The amounts of *C. burnetii* in smear preparations were evaluated in crosses as described (Kazár *et al.*, 1973). Twenty per cent individual or pooled organ suspensions of the same type from the same animal species and group in BHI were prepared and stored at −30 °C. They were bioassayed in guinea pigs and mice by determination of serum phase II antibody response 4 weeks after i.p. inoculation or in chick embryos by detection of *C. burnetii* in yolk sac impression smears on day 10 p.i.

Serological examinations. Guinea pig and mouse sera harvested at intervals after *C. burnetii* infection and after immunization with Q fever phase I corpuscular vaccine were examined by the MA test according to Fiset *et al.* (1969) with stained corpuscular phase I antigen and with stained artificial phase II antigen prepared as described (Schramek *et al.*, 1972). Sera of guinea pigs and mice inoculated with tested organ suspensions were examined only with artificial phase II antigen. Titres $\geqq 8$ were considered as positive.

Results

Distribution of C. burnetii in organs of mice and guinea pigs following i.p. infection

To decide which organs should be chosen for examination of the presence of *C. burnetii* in immunized and non-immunized animals, mice and guinea pigs were infected i.p. with 10^6 and 10^4 EID_{50} of *C. burnetii*, respectively. Along with the examination of amounts and yields of *C. burnetii* in organs tested, the phase I and phase II MA antibody responses were examined.

Table 1 presents the results of distribution of *C. burnetii* in mouse organs. In early stages of infection (up to the 14th day), rickettsiae were detected in all organs tested, highest amounts and yields being found in spleen and liver. In the latter organs, *C. burnetii* could be detected also at later intervals by both light microscopy and IF, but no rickettsiae were isolated in chick embryo yolk sacs. Continual presence of *C. burnetii* was detected microscopically in the peritoneum, but live rickettsiae were not determined in the peritoneal samples. The blood clearance of *C. burnetii* on day 14 p.i. coincided with appearance of serum phase I MA antibodies. At later intervals, when higher phase I MA antibody titres were found, *C. burnetii* disappeared also from other organs tested, except kidneys and reproductive tract, in which *C. burnetii* was hardly detectable microscopically, but had to persist, because inoculation of their suspensions into chick embryo yolk sac yielded live rickettsiae. Similar results were obtained in experiments on guinea pigs, in which again kidneys and reproductive organs represented the only tissues tested for persisting live *C. burnetii*.

Based on these results, kidneys and reproductive organs along with the spleen and blood were chosen to test the presence of *C. burnetii*.

Persistence of C. burnetii and its reactivation by cortisone- or CPA-treatment in non-vaccinated and vaccinated guinea pigs

Guinea pigs infected for 6 weeks with 10^4 EID_{50} of *C. burnetii* were non-immunized or immunized with 500 μg of Q fever phase I corpuscular vaccine and treated with cortisone or CPA at intervals and with doses as described above. Individual 20% suspensions of the blood, spleen, kidneys and reproductive organs prepared from three guinea pigs of each group were inoculated i.p. into 4 guinea pigs and 4 mice serving as indicator animals. After 4 weeks, surviving 3—4 mice or 2—3 guinea pigs in groups given the same material were bled, their sera were pooled and tested for presence of phase II MA antibodies. In parallel, we examined sera of indicator animals inoculated with organ suspensions from non-infected untreated guinea pigs and with Q fever vaccine only to determine whether residual *C. burnetii* resulting from Q fever phase I corpuscular immunization may influence the antibody response.

As follows from Table 2, no antibody response was found in indicator animals inoculated with the blood suspensions. The antibody response in animals inoculated with the spleen suspensions was irregular in control and

Table 1. Distribution of C. burnetii in mouse organs following i.p. infection

Days p.i.	Organs									MA antibody titres	
	spleen	liver	kidney	lung	brain	lymph nodes	blood	reprod. organs	perito-neum	phase I	phase II
3	++++[a]5.6[b]	+++ 4.6	+ 2.8	(+)2.7	(+)1.8	+ 2.6	+ 3.1	++ 2.6	++ n.t.	<2	<2
7	+++ 4.7	++ 4.1	+ 2.6	(+)2.5	(+)1.5	(+)1.6	+ 1.8	+ 2.1	++ n.t.	<2	64
14	+++ 2.8	++ 2.1	+ 2.0	(+)1.3	(+)1.2	(+)1.3	(+)neg.	+ 1.8	+ n.t.	8	1024
21	++ neg.	++ neg.	+ 1.8	− neg.	− neg.	(+)neg.	− neg.	+ 1.6	+ n.t.	32	4096
28	++ neg.	+ neg.	(+)1.6	− neg.	− neg.	− neg.	− neg.	(+)1.5	(+)n.t.	128	4096
42	+ neg.	(+)neg.	(+)1.3	− neg.	− neg.	− neg.	− neg.	(+)1.2	(+)n.t.	64	2048

[a] Amount of C. burnetii in impression smears as evaluated by light microscopy and IF: − no rickettsiae, (+) single rickettsiae, + less than ten, ++ tens, +++ hundreds, and ++++ uncountable number of rickettsiae per each of 10 different fields of view.

[b] Yield of *C. burnetii* (log EID_{50}/0.25 ml units) from 20% pooled organ suspensions titrated in chick embryo yolk sacs. n.t. = not teste; neg. = negative.

Table 2. Phase II MA antibody response[a] in mice (M) and guinea pigs (Gp) inoculated with organ suspensions of C. burnetii-infected guinea pigs immunized with Q fever phase I corpuscular vaccine

Group of guinea pigs	Organ suspension samples							
	Blood		Spleen		Kidney		Reproductive organs	
	M	Gp	M	Gp	M	Gp	M	Gp
Control	0	0	0	0	2	3	1	1
Im-control	0	0	0	1	1	3	0	1
Cortisone	0	0	1	0	2	3	1	1
Im-cortisone	0	0	0	0	1	3	1	1
CPA	0	0	2	2	3	3	1	2
Im-CPA	0	0	1	1	3	3	1	1

[a] The presence of antibody response in pooled sera of 3—4 mice and 2—3 guinea pigs, respectively, expressed as the number positive per total of 3 individual suspensions of each guinea pig organ tested.

cortisone-treated groups, but not in the group of CPA-treated guinea pigs. When comparing suspensions of kidney and reproductive organs, the former elicited antibody response more regularly, guinea pigs being more susceptible. The phase II MA antibody titres varied from 8 to 2048 in all groups tested. Antibody response was less frequent when testing organ suspensions from guinea pigs given Q fever vaccine (whether they were untreated or treated with cortisone or CPA), indicating some but incomplete effect of

Table 3. Phase II MA antibody response[a] in mice (M) and guinea pigs (Gp) inoculated with organ suspensions of 6-week C. burnetii-infected mice immunized with Q fever phase I corpuscular vaccine and treated with cortisone or CPA or becoming pregnant during the experiment

Group of mice	Organ suspension samples							
	Blood		Spleen		Kidney		Reproductive organs	
	M	Gp	M	Gp	M	Gp	M	Gp
Control	0	0	0	0	1	1	1	1
Im-control	0	0	0	1	0	1	2	1
Cortisone	1	1	1	2	2	3	0	1
Im-cortisone	1	1	0	1	1	3	0	0
CPA	1	2	1	2	1	3	2	1
Im-CPA	0	1	1	3	1	3	1	1
Pregnant	0	0	1	2	2	2	1	1
Im-pregnant	0	0	1	2	2	2	1	2

[a] The presence of antibody response (expressed as the number positive per total of 3 in each group) in individual sera of 3 mice or guinea pigs 4 weeks after inoculation with pooled suspensions of the mouse organs.

Table 4. The amounts of C. burnetii in suspensions of kidney and reproductive organs of one month-C. burnetii-infected mice immunized with Q fever phase I corpuscular vaccine and treated with cortisone or CPA

Group of mice	Antibody response[a] in mice					Antibody response[a] in guinea pigs					The presence of C. burnetii[b] in chick embryo yolk sacs inoculated with				
	K0*	K1	K2	R0*	R1	K0	K1	K2	R0	R1	K1	K2	K3	R1	R2
Control	4	4	2	2	1	2	1	0	2	1	4	1	0	1	0
Im-control	4	4	2	3	2	3	1/2	1	1	1	2	1	0	1	0
Cortisone	4	4	4	4	3	3	2/2	2/2	3	1	6	2	0	3	0
Im-cortisone	4	4	3	4	1	2/2	2	1	3	1	1	1	0	1	0
CPA	4	4	4	4	4	2/2	2/2	2	2/2	2/2	n.t.	3/5	3	4/6	4
Im-CPA	4	4	2	4	3	2/2	2	1	1/1	2	n.t.	1/5	0	3	1

[a] The presence of antibody response expressed as the number positive per total of 4 mice an 3 quinea pigs, respectively, or as the number positive/number of inoculated animals.
* Symbols represent tenfold dilution of kidney (K) or reproductive organ (R) suspensions tested.
[b] The presence of C. burnetii expressed as the number positive per total of 8 chick embryo yolk sacs, or as the number of positive/number of inoculated chick embryo yolk sacs.
n.t. = not tested, because chick embryos died after inoculation.

vaccination on the persistence of *C. burnetii* and on its reactivation. Because low antibody response (titre of 8) was observed only in the case of spleen suspension from guinea pigs inoculated with Q fever vaccine alone, this vaccine could influence the antibody response resulting from inoculation of spleen, but not of other organ suspensions tested.

Examination by IF revealed variable presence of *C. burnetii* in individual organ suspensions, the kidneys and reproductive organs of cortisone- or CPA-treated non-immunized guinea pigs being more regularly positive. In guinea pigs inoculated with Q fever vaccine alone, *C. burnetii* could be detected only in spleen suspensions.

Persistence of C. burnetii and its reactivation by treatment with cortisone or CPA or by parturition in non-vaccinated and vaccinated mice

Mice were infected with 10^6 EID_{50} of *C. burnetii* 6 weeks before they were immunized with 100 μg of Q fever phase I corpuscular vaccine; then the immunized as well as non-immunized animals were treated with cortisone or CPA at intervals and with doses described above. Twenty per cent suspensions of blood, spleen, kidneys and reproductive organs were pooled from 4 to 6 mice of each group. In parallel, organ suspensions prepared from mice inoculated with Q fever vaccine alone, and from mice which became pregnant about 6 weeks after infection with *C. burnetii*, i.e. at the time of administration of Q fever vaccine. Pooled organ suspensions were inoculated i.p. into 6 mice and 3 guinea pigs serving as indicator animals. Sera of 3 guinea pigs were examined individually, sera of two mice were pooled and

Table 5. The amounts of C. burnetii in suspensions of kidneys and reproductive organs of 6 month-C. burnetii-infected guinea pigs immunized with Q fever phase I corpuscular vaccine and treated with cortisone or CPA

Group of guinea pigs	Antibody response[a] in mice					in guinea pigs					The presence of C. burnetii[b] in chick embryo yolk sacs inoculated with				
	K0	K1	K2	R0	R1	K0	K1	K2	R0	R1	K1	K2	K3	R1	R2
Control	4	4	2	4	2	3	2	1	2	2	4	2	0	2	0
Im-control	4	4	0	4	3	1/2	1/2	0	1	1	2	1	0	2	0
Cortisone	4	4	3	4	4	2/2	3	2	2/2	2	5	2	0	3	0
Im-cortisone	4	2	1	4	1	2/2	3	1	3	1	1	1	0	1	0
CPA	n.t.	2/2	4	n.t.	4		3	2/2	2/2	2	n.t.	2/3	1/4	3/6	1
Im-CPA	3/3	2/2	3	4	2/2	2/2	2	1	2/2	2	3/4	1/3	0/4	1/6	0

For explanations see legend to Table 4.

examined, so that in each group three samples of mouse and guinea pig sera were evaluated.

As shown in Table 3, *C. burnetii* persisted mainly in kidneys and reproductive organs; its persistence was again only variably influenced by Q fever vaccine administration. The vaccination did not substantially affect reactivation of *C. burnetii* by treatment with cortisone or CPA, and by parturition, respectively. Residual *C. burnetii* resulting from administration of the vaccine alone might influence the results obtained only in those indicator animals which were inoculated with spleen suspensions. Results of IF examination of organ suspensions were similar to those observed in guinea pigs.

Effect of Q fever phase I corpuscular vaccine on C. burnetii in kidneys and reproductive tract of infected mice and guinea pigs untreated or treated with CPA or cortisone

Mice infected for one month with 10^6 EID_{50} and guinea pigs infected for 6 months with 10^4 EID_{50} of *C. burnetii*, respectively, were given Q fever phase I corpuscular vaccine and remained untreated or were treated with cortisone or CPA as described above. Individual 20% suspensions from kidneys and reproductive tract of 6 mice and 4 guinea pigs were prepared and tested for bacterial sterility. Sterile suspensions pooled from 3 to 4 guinea pigs and from 4 to 6 mice in each group were ten-fold diluted and inoculated into 8 chick embryo yolk sacs, into 4 mice and 3 guinea pigs i.p. The latter were tested individually to determine the antibody response to *C. burnetii*.

As follows from Tables 4 and 5 all organ suspensions contained *C. burnetii*, irrespective of whether they were prepared from immunized or non-immunized, treated or untreated animals. In general, more *C. burnetii* were detected in organs of treated (namely by CPA) than of untreated animals,

Table 6. The MA antibody response in 6 week-C. burnetii-infected guinea pigs and mice given Q fever phase I corpuscular vaccine and treated with cortisone or CPA

Group of animals	Mean geometric titres[a] of MA antibodies in sera[b] of guinea pigs		mice	
	phase I	phase II	phase I	phase II
Control	10	40	42	294
Im-control	294	891	91	575
Cortisone	10	74	67	388
Im-cortisone	315	722	147	512
CPA	5	14	23	128
Im-CPA	14	84	45	215
Uninfected Q fever vaccine only	64	256	86	340

[a] Mean geometric titres calculated from 4 guinea pig and 6 mouse sera, respectively.
[b] Sera taken 18 days after administration of Q fever phase I corpuscular vaccine.

and less *C. burnetii* in organs of animals immunized with Q fever vaccine. Thus, vaccination could reduce the amount of *C. burnetii* in the organs tested, but was unable to free them from the agent.

The MA antibody response in infected guinea pigs and mice inoculated with Q fever phase I corpuscular vaccine and treated with cortisone or CPA

To determine whether humoral immunity could be affected by treatment with cortisone or CPA in guinea pigs and mice which were or were not immunized with Q fever phase I corpuscular vaccine 6 weeks after their infection with *C. burnetii*, phase I and phase II antibody response was determined by MA test in sera harvested one day after the end of drug treatment (18 days after Q fever vaccine administration) and mean geometric antibody titres were calculated. The MA antibody response was followed also in sera of non-infected and untreated guinea pigs and mice 18 days after inoculation of Q fever vaccine alone. As shown in Table 6, an anamnestic response occurred after administration of Q fever vaccine as demonstrated by an increase in mean geometric titres of both phase I and phase II antibodies. This response was higher in guinea pigs than in mice. In animals treated with cortisone an augmentation rather than an inhibition of phase I and phase II antibody response was observed in infected as well as in infected and immunized animals. Treatment with CPA resulted in significantly lower phase I and phase II MA antibody titres in either group of animals tested.

Discussion

The tendency of *C. burnetii* to persist in the host tissues is well known. It sems probable that such persistence represents some kind of steady state between the host and the agent. The steady state can be affected in favour

of the infectious agent by pregnancy and parturition (Sidwell and Gebhardt, 1966), by whole body X-irradiation (Sidwell *et al.*, 1964*a*) as well as by cortisone (Sidwell *et al.*, 1964*b*) or CPA treatments (Tokarevich, 1979). These procedures may interfere with the immune response of *C. burnetii*-infected host resulting in the reactivation of infection. In pregnant hosts effects of hormones and stress applied to the reproductive tract tissue may also aid in the reactivation process (Sidwell and Gebhardt, 1966). As to the humoral immunity, irradiation and multiple cortisone injections in white mice, deer mice and guinea pigs caused variation of Qfever antibody response (Sidwell *et al.*, 1964*a*, 1964*b*). Activation of *C. burnetii* in guinea pigs by CPA was accompanied by a decrease in IgG and a relative increase in IgM antibodies (Tokarevich, 1979). In our study, treatment with cortisone rather increased than decreased phase I and phase II antibody responses, but these were reduced markedly in the groups of CPA-treated mice and guinea pigs. However, studies in such a way treated or in pregnant animals should be extended to the evaluation of cell-mediated immune responses which play a crucial role in immunity to *C. burnetii* infection (Jerrells *et al.*, 1975; Hinrichs and Jerrells, 1976; Kishimoto *et al.*, 1978; Kazár *et al.*, 1982).

One can speculate that administration of Q fever vaccine as secondary antigenic impulse may disbalance the steady state between persisting *C. burnetii* and the host in favour of the host. This idea is supported by results of studies with *Brucella abortus*, the agent similar to *C. burnetii* in its ability to cause persistent infection which can be reactivated during parturition, e.g. injection of dead Brucella during the latent phase of a Brucella infection caused a sudden drop in the number of residual brucellae persisting in the tissues (Mackaness and Blanden, 1967) and recall of acquired cellular resistance in mice by antigens from killed Brucella was observed (Halliburton and Hindsdill, 1972). In the case of rickettsiae such an effect can be explained by activation of macrophages by killed *C. burnetii* cells (Kelly, 1967) or by the inhibitory effect of lymphokines on multiplication of *C. burnetii* on macrophages (Hinrichs and Jerrells, 1976), when taking into consideration the possibility of macrophage inactivation for rickettsial killing by lymphokines added to macrophages after infection with *Rickettsia tsutsugamushi* (Nacy and Meltzer, 1979). As follows from our study, such an impulse reducing the number of *C. burnetii* in animal tissues was not effective enough to clear them from *C. burnetii* completely.

Of interest from the theoretical point of view is the distribution of *C. burnetii* in tissues of i.p. infected guinea pigs and mice. The rapid clearance of *C. burnetii* within three weeks p.i. from the spleen and liver, which is in accord with an observation of others (Hinrichs, personal communication) contrasted with its persistence in kidneys and reproductive tract as observed also by Sidwell *et al.* (1964*a*). It has important practical consequences, because of the possibility of shedding of *C. burnetii* via urine or placental tissues. It is unknown, whether it could be attributed to some favourable conditions for persistence and multiplication of *C. burnetii* in kidneys and reproductive tract or the relative absence in these organs as compared to

the spleen and the liver of phagocytic cells, namely macrophages, which might be responsible for destruction of *C. burnetii*. On the other hand, macrophages were implicated as the cells in which *C. burnetii* could persist (Khavkin and Amosenkova, 1969) parasitising in their phagolysosomes (Ariel *et al.*, 1973).

The original purpose of our study was to throw light on the possibility of prevention by Q fever phase I corpuscular vaccine of *C. burnetii* persistence, reactivation and shedding, respectively, in animals infected previously with *C. burnetii*. Such a prevention of shedding *C. burnetii* via milk and placental tissues of naturally infected dairy cows remains a matter of controversy, though the prophylactic value of Q fever vaccine has been generally admitted (Biberstein *et al.*, 1977; Sádecký *et al.*, 1974; Sádecký *et al.*, 1975; Sádecký, 1978; Schmittdiel *et al.*, 1981). We are far from mechanical application of our results from experiments on laboratory animals to naturally infected domestic animals. Nevertheless, administration of Q fever phase I corpuscular vaccine affected neither the persistence of *C. burnetii* in the mouse and guinea pig kidneys and reproductive tract, nor the reactivation of *C. burnetii* infection by parturition or by treatment with cortisone or CPA, which should be taken into account when considering vaccination against Q fever of animals previously infected with *C. burnetii*.

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