J.C. Williams [1]

M.G. Peacock[2]

R.E. Race [2]

Immunization of dogs with Q fever vaccines : comparison of phase I, II and phase I CMR *Coxiella burnetii* vaccines

WILLIAMS (J.C.), PEACOCK (M.G.), RACE (R.E.). Immunisation de chiens avec des vaccins contre la fièvre Q : comparaison entre des vaccins de *Coxiella burnetii* de phase I, phase II et du RCM de phase I. *Revue Élev. Méd. vét. Pays trop.*, 1993, **46** (1-2) : 87-94

Des vaccins contre la fièvre Q ont été testés sur des chiens de races croisées en utilisant des cellules entières de *Coxiella burnetii* inactivées à la formaline dans la phase I (CEI) ou la phase II (CEII), ou le résidu obtenu par extraction par chloroforme/méthanol (RCM) de cellules en phase I. Le vaccin CEI mélangé (1:1) à l'adjuvant incomplet de Freund (AIF) a provoqué des réponses immunitaires humorales aux antigènes des phases I et II, comme il a été mesuré par le test de microagglutination. Le vaccin RCM mélangé (1:1) à l'AIF a engendré des titres d'anticorps spécifiques aux antigènes de phases I et II plus élevés que le vaccin CEI. Le vaccin CEII a produit seulement des anticorps contre des antigènes de phase II. La durée d'un érythème et d'une induration, après des tests dermiques avec des antigènes de *Coxiella burnetii,* fait penser à une immunité cellulaire. Bien que des granulomes aient été observés, seulement avec les vaccins CEI et CEII, aucun des antigènes utilisés dans le test dermique n'a provoqué des abcès aux points d'injection. En revanche, les ganglions axillaires qui drainent le point d'injection des vaccins ont développé chez tous les chiens des abcès stériles drainants après 19 à 24 jours pour les vaccins CEI et RCM, et 104 jours pour le vaccin CEII. Les abcès se sont résolus moins de 30 jours après leur première apparition. Les réponses des lymphocytes du sang, des ganglions axillaires et mésentériques et de la rate, au Con A, à la PHA et aux antigènes utilisés, 222 jours après la vaccination, étaient variables. Les lymphocytes des organes divers ont répondu à un ou plus des antigènes de rappel et aux deux mitogènes, en l'absence ou la présence d'indométhacine. Bien que ces vaccins contre la fièvre Q aient provoqué une immunité humorale et cellulaire, des abcès stériles drainants ont été provoqués, soit par les antigènes, soit par l'AIF. Les résultats des tests dermiques font penser que le vaccin RCM est le meilleur choix comparé aux vaccins CE, étant donné l'absence de formation tardive de granulomes par le premier. D'autres études seront nécessaires pour déterminer l'origine des réactions indésirables et pour évaluer l'efficacité des vaccins contre la coxiellose des chiens.

Mots clés : Chien - *Coxiella burnetii* - Fievre Q - Vaccin - Immunisation - Réponse immunitaire - Technique immunologique - Immunité cellulaire.

1. Food and Drug Administration, Center for Biologics Evaluation and Research, Office of Vaccine Research and Review, Division of Vaccines and Related Products Applications, Bacterial Vaccine and Allergenic Products Branch, HFM-481 Woodmont 400N, 1401 Rockville Pike, Rockville, MD 20852-9090, Etats-Unis.

2. National Institute of Allergy and Infectious Diseases, Rocky Mountain Laboratories, Hamilton, Montana 59840, Etats-Unis.

This study was conducted at the Rocky Mountain Laboratory, NIAID, Hamilton, Montana, Etats-Unis.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals", as prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources Commission of Life Sciences-National Research Council. The facilities are fully accredited by the American Association for accreditation of Laboratory Animal Care.

INTRODUCTION

Coxiella burnetii causes Q fever in humans (3) and coxiellosis in animals (5). Coxiellosis in companion animals and livestock is a risk factor for the acquisition of Q fever (23). Epidemiologic and epizootiologic studies show asymptomatic and/or clinical cases of Q fever to be sporadic and linked with exposure to domestic animals (7, 11). Human infection is acquired from aerosols of *C. burnetii* during parturition in domestic animals such as Bovidae *(Bos taurus* (cattle), *Capra hircus* (goat), *Ovis aries* (sheep)) (17), Felidae *(Felis domesticus* (cat)) (9), and of Canidae *(Canis familiaris* (dog)) after eating the liver of Cervidae (deer) (6), and of wild Leporidae (rabbit) (10). Among pet owners and their contacts airborne Q fever often accounts for significant morbidity and occasional mortality in humans residing in urban, rural and feral settings.

Vaccination of humans and animals is recommended for the prevention and control of Q fever (12) because of the ubiquity of *C. burnetii* in wild and domestic animals. The immunization of humans with formalin-inactivated phase I *C. burnetii* (Henzerling strain) is efficacious (8), but booster injections cannot be safely given because of the likely induction of granuloma which may form sterile abscesses. Various Q fever vaccines are effective in decreasing the shedding of C. *burnetii* in milk, birthing fluids and tissues of animals (13). In this report, we have compared the immunogenicity and pathogenicity of phase I whole-cell (WCI), phase II WC (WCII), and phase I CMR vaccines in mixed breed dogs.

MATERIALS AND METHODS

Vaccines

Coxiella burnetii were grown in the yolk-sac of fertile hen's eggs, separated from host components, inactivated with formalin and prepared as vaccine (20, 21). Extraction of lyophilized phase I Ohio WC with C:M (4:1) was done to produce the CMR vaccine (19).

Immunization

Mixed breed dogs (3 male and 3 female) weighing roughly 23±2 kg were pre-bled and injected with vaccine as follows. On day 0, six dogs (two per vaccine) were injected i.v. with 50 µg (dry weight) of WCI or WCII or CMR in 1 ml of saline, and 50 µg (2 x 25 µg) s.c. in the region of the axillary nodes with vaccine suspended (1:1) in Freund's incomplete adjuvant (FIA) (Difco Laboratories, Detroit, MI, USA). On day 14, each dog was bled and injected i.m. in the left rear upper leg with 100 µg of homologous vaccine suspended in FIA. Animals were evaluated daily for signs of adverse reactions (*i.e.*, physical abnormalities, erythema, induration and abscess) at the injection site up to 221 days following vaccination.

Animals, previously fasted for 24 hours, were anesthetized with sodium pentobarbital (Nembutal sodium-Abbott, Chicago Ill.) administered intravenously (50 mg/5 lbs body weight). Three-fourths of the calculated dose was given rapidly and the rest as needed to induce a surgical plane of anesthesia. The animals necks were shaved and then thoroughly scrubbed with Phisohex disinfectant soap and alternately swabbed several times with 95 % alcohol and zephiran chloride (Sterling Drug Co. New York, N.Y. USA). Blood was then collected and the dogs simultaneously exsanguinated by jugular transection. Prescapular and mesenteric lymph nodes and spleen sections were immediately obtained aseptically. About one gram of each tissue was dissociated by carefully forcing the cells through a stainless steel screen while immersed in physiologic buffered balanced salt solution (PBBS). The cells were washed three times with PBBS and viability determined by trypan blue exclusion. These unfractionated cells were adjusted to a concentration of 5 x 10^6/ml in RPMI 1640 (Grand Island Biological Co., Grand Island, New York) containing 5 % autologous serum and 1 µg/ml gentamicin.

Immunological assays

Humoral anti-*C. burnetii* antibodies were evaluated by a microagglutination assay (MAA) (4). The response of splenic and nodal (axillary and mesenteric) lymphocytes to recall antigens (WCI, WCII, and CMR at 0, 1, 10, and 50 µg per ml) were evaluated. Whole blood was diluted 1:30, a dilution previously determined to be optimal (data not shown), in the RPMI medium without serum and containing 1 µg/ml gentamicin. The effect of indomethacin (IND) (16) (1, 10, and 50 µg per ml) on dog lymphocyte proliferation assay (LPA) was assessed in the absence and presence of the recall antigens, and in the absence and presence of concanavalin A (Con A) (0.1, 1, and 10 µg per ml), and phytohemagglutinin (PHA)(1:20, 1:200, and 1:400 dilution) at 37°C in a 5 % CO_2 incubator for 5 days. After 4 days incubation in microtiter plates, 1 µCi of [^{3}H]TdR (specific activity, 5 Ci/mmol ; Amersham Corp., Arlington Heights, Ill.) was added to each well and incubation was continued for another day. [^{3}H]TdR labelled DNA was collected on micro-fiber class filters and the CPM per filter was determined.

Responses of nucleated cells under various experimental conditions were expressed as stimulation indices (SI) as follows : SI = (CPM in IND and/or mitogen and/or recall antigen stimulated cells ÷ CPM in unstimulated cells - background CPM).

Skin test procedure

Dermal hypersensitivity testing consisted of the i.d. injection of 0.1 ml of vaccine at various dilutions. The right side of each dog was shaved from the midline between the legs and down the side to a position that accommodated three rows of the diluted vaccines. Animals vaccinated with WCI or CMR were skin tested with 10, 1, 0.1, and 0.01 µg of WCI and CMR, and 100, 10, 1, and 0.1 µg of WCII. Animals vaccinated with WCII were skin tested with only WCII at 100, 10, 1, and 0.1 µg. The diameter in mm of erythema was measured with a ruler. The skin thickness in mm of induration was measured with a skin calliper.

RESULTS

Adverse reactions

Between days 19 and 24 after the injections of the WCI or CMR vaccines, the dogs began limping on their left rear leg. Significant swelling (i.e. ≥ 5 mm) was noted at the i.m. and s.c. injection sites. Slight erythema was present over the injection sites. Animals injected with WCII developed similar lesions 104 days afterwards. Within another 24 h after observing the lesions all of the swollen areas had formed abscesses, which erupted and began to drain. The fluid collected from each of the abscesses did not have an odor and no microorganisms were observed by standard bacteriological techniques. To prevent secondary infection, all of the animals were treated with injectable penicillin and streptomycin. The lesions resolved spontaneously within 30 days.

Humoral immune response

The temporal sequences of anti-phase I and anti-phase II antibodies after the injections of vaccine were compared (table I). Animals did not have titers to the antigens prior to vaccination. WCI vaccinated animals developed antigen specific antibodies to both phase I and phase II antigens. Application of the skin test antigens caused an increase in antiphase II antibodies in only one dog. WCII vaccinated animals developed antigen-specific antibodies

TABLE I Humoral immune response of mixed breed dogs to Q fever vaccines.

Vaccine	Days after vaccination and skin test[a]		Microagglutination titer			
			PhII	PhI	PhII	PhI
	Dog 1	*Dog 2*	*Dog 1*		*Dog 2*	
Phase I	0	0	< 2	< 2	< 2	< 2
	14	14	128	128	128	64
	24	24	128	128	256	128
	32	32	128	128	128	128
	95	95	32	64	32	64
	220[b]	220	32	32	64	64
	227	227	32	64	64	64
	234	234	64	64	256	64
	Dog 3	*Dog 4*	*Dog 3*		*Dog 4*	
Phase II	0	0	< 2	< 2	< 2	< 2
	14	14	512	< 2	1024	< 2
	24	27	512	< 2	512	< 2
	32	125[c]	512	< 2	64	< 2
	95	132	8	< 2	64	< 2
	220[c]	138	16	< 2	512	< 2
	227		32	< 2		
	234		64	< 2		
	Dog 5	*Dog 6*	*Dog 5*		*Dog 6*	
CMR	0	0	< 2	< 2	< 2	< 2
	14	14	256	32	1024	16
	27	27	128	32	2048	1024
	125[b]	125	128	4	256	16
	132	132	64	64	128	64
	138	138	512	256	512	1024

[a] *Day that animals were bleed for the detection of humoral immune responses to* Coxiella burnetii.
[b] *Animals skin tested with 11.1 µg phase I, 111.1 µg phase II and 11.1 µg CMR.*
[c] *Animals skin tested with 111.1 µg phase II.*

to only phase II antigen. Application of the skin test antigen caused a marked increase in anti-phase II antibodies in both dogs. CMR vaccinated animals developed antigen-specific antibodies to both phase I and phase II antigens. The antibody titers induced by the CMR vaccine were markedly greater than those induced by the WCI vaccine. Application of the skin test antigens caused a marked increase in both anti-phase I and anti-phase II antibodies in both dogs.

In vitro cellular immune responses

Cellular immune responses were evaluated in vitro 221 days after vaccination. The effect of IND (0, 1, 10, 50 µg/ml) on the lymphocytes of a normal dog was determined in the presence of Con A (0, 0.1, 1.0, 10 µg/ml), PHA (0, 1:20, 1:200, 1:400), WCI, WCII and CMR (all antigens at 0, 1, 10, 50 µg/ml).

Blood lymphocytes

The normal dog lymphocytes responded optimally to the mitogens, Con A and PHA, and IND at 1.0 µg, 1:20 dilution and 1.0 µg, respectively. The mitogenic activity of the antigens revealed no change from 0 to 50 µg for WCI in the presence or absence of IND, but both CMR and WCII induced an optimum response at 1.0 µg with IND at 1.0 µg. The lymphocytes from vaccinated animals responded optimally with the same concentrations of mitogen and antigen combinations.

Splenic and lymph node lymphocytes

The normal dog lymphocytes responded optimally to the mitogens, Con A and PHA, and IND at 1.0 µg, 1:20 dilution, and 1.0 µg, respectively. The antigens had optimal mitogenic activity at 1.0 µg in the presence of IND at 1.0 µg. The lymphocytes from vaccinated animals responded

similarly to mitogen and antigen combinations. Was compared the SI for each of the animal's lymphocytes without IND and with 1.0 µg IND (table II). Although the lymphocytes from each vaccinated animal responded with different SI values to mitogens and antigens in the presence or absence of IND, the animal's lymphocytes responded with a 2-fold or greater increase in activity to at least one of the recall antigens. The observed variability between dog lymphocytes and organs was expected from these mixed breed dogs.

TABLE II Comparison of **in vitro** *cellular immune response of dogs vaccinated with Q fever vaccines*

Animal mitogen or antigen	Response as SI without/with indomethacin			
	Whole blood	Spleen	Axillary nodes	Mesenteric nodes
Control				
Con A	38.4 / 81.5	22.4/24.5	66.4/99.7	242.8/348.7
PHA	13.4/37.4	15.9/28.5	19.8/23.7	172.8/181.4
CBOI	1.2/1.3	0.3/2.8	1.1/3.0	1.1/1.7
CMRI	0.6/1.2	0.5/4.7	1.1/4.1	1.2/1.3
Phase II	0.7/1.1	0.4/1.8	0.7/1.6	1.1/0.9
Phase I Vac				
Con A	15.7/16.5	6.4/1.1	40.5/158.1	265.5/211.5
PHA	8.1/10.1	10.0/4.6	41.0/74.2	187.6/67.6
CBOI	0.7/1.0	5.2/4.2	5.6/5.3	2.5/1.0
CMRI	0.7/1.0	6.3/6.9	7.4/4.8	3.3/0.8
Phase II	0.6/1.0	4.0/3.9	3.7/6.7	0.8/1.3
Phase I Vac				
Con A	5.9/1.3	30.5/1.2	4.7/0.3	70.3/0.9
PHA	4.0/1.1	0.6/0.6	4.0/1.3	10.5/0.7
CBOI	0.4/0.5	0.4/1.1	1.4/1.0	2.0/1.6
CMRI	1.0/0.4	0.3/1.4	0.7/0.9	0.9/2.1
Phase II	0.7/0.5	0.9/0.9	1.0/1.3	1.0/1.1
CMR Vac				
Con A	15.1/18.1	0.4/2.5	62.4/55.9	85.9/290.4
PHA	10.9/6.7	3.2/6.5	36.5/38.8	73.1/155.6
CBOI	0.5/1.2	3.3/4.2	3.3/2.4	0.5/1.8
CMRI	0.6/0.9	3.6/5.0	3.3/2.6	0.8/1.8
Phase II	0.7/0.4	2.1/6.0	3.7/2.9	0.8/1.0
Phase II Vac				
Con A	10.7/14.4	22.0/6.5	114.9/67.1	339.1/338.4
PHA	9.8/9.6	19.1/8.5	27.0/18.0	78.1/48.7
CBOI	0.4/1.2	2.0/1.5	1.2/0.5	0.9/0.6
CMRI	0.6/0.3	6.4/2.4	1.0/0.8	0.5/1.0
Phase II	0.4/1.8	1.2/2.2	2.6/1.0	1.0/0.6
Phase II Vac				
Con A	18.8/0.2	6.6/1.0	36.6/0.9	54.6/1.1
PHA	7.0/1.1	2.2/0.9	11.6/1.1	10.2/2.2
CBOI	0.7/1.1	1.9/1.0	0.8/1.3	1.1/1.2
CMRI	0.6/1.3	1.0/1.0	1.2/1.0	0.3/1.1
Phase II	0.5/1.1	0.5/1.0	0.6/0.8	1.3/0.7

In vivo skin test responses

As a test of their ability to elicit skin test responses, the vaccine antigens were tested in each of the dogs (tables III to VII). No erythema was observed when saline was used at the injection site. Therefore, any erythematous reaction was graded as positive. Because of the variation in skin thickness measurements any change of ≥ 20 mm was graded as significant. Animals vaccinated with WCI showed early (4 h) and late (96 h) erythema, and only late induration responses to all three skin test antigens. Animals vaccinated with WCII showed early and late erythema, and only late induration responses to WCII skin test antigen. The other two antigens were not tested in WCII vaccinated animals because the authors did not want to complicate the humoral immune response profile by injecting animals with phase I antigens. Animals vaccinated with CMR showed only early erythematous, and only early induration responses, but no late responses with all three skin test antigens.

TABLE III Comparison of skin test reponses in dog No. 1 vaccinated with the WCI vaccine.

Hour after skin test	Skin test antigen	Response (mm erythema/induration)			
		10	1	0.1	0.01
0	PHASE I	0/73	0/72	0/90	0/130
4		21/75	0/75	0/93	0/130
24		0/86	0/79	0/90	0/130
48		0/79	0/75	0/91	0/130
72		0/80	0/79	0/86	0/130
96		30/99	0/94	0/84	0/130
120		0/84	0/84	0/93	0/130
0	PHASE II*	0/69	0/69	0/75	0/81
4		15/74	0/77	0/79	0/103
24		0/82	0/79	0/79	0/86
48		0/76	0/71	0/77	0/96
72		0/84	0/76	0/78	0/93
96		150/130	72/100	0/83	0/85
120		0/101	0/85	0/83	0/85
0	CMR	0/57	0/66	0/70	0/85
4		77/74	0/79	0/78	0/88
24		0/79	0/76	0/81	0/96
48		0/69	0/69	0/77	0/93
72		0/76	0/79	0/81	0/94
96		156/130	49/76	0/85	0/83
120		0/117	0/86	0/83	0/74

* *The skin test dose was 100, 10, 1, and 0.1 rather than 10, 1, 0.1, and 0.01 µg.*

TABLE IV Comparison of skin test reponses in dog No. 2 vaccinated with the WCI vaccine.

Hour after skin test	Skin test antigen	Response (mm erythema/induration)			
		10	1	0.1	0.01
0	PHASE I	0/45	0/45	0/45	0/53
4		100/56	104/59	36/60	0/78
24		150/49	49/49	0/46	0/69
48		0/47	0/49	0/57	0/69
72		0/52	0/49	0/52	0/71
96		140/64	0/61	0/56	0/69
120		0/54	0/55	0/53	0/66
0	PHASE II*	0/42	0/42	0/45	0/83
4		100/54	216/55	0/56	0/98
24		0/49	0/42	0/46	0/68
48		0/47	0/45	0/50	0/63
72		0/59	0/50	0/51	0/69
96		110/65	0/52	0/52	0/53
120		0/57	0/46	0/46	0/49
0	CMR	0/36	0/36	0/42	0/44
4		110/57	156/58	12/50	6/50
24		9/43	84/48	0/46	0/50
48		0/46	0/47	0/45	0/46
72		0/52	0/46	0/46	0/51
96		156/71	49/61	0/52	0/51
120		0/51	0/62	0/53	0/43

* *The skin test dose was 100, 10, 1, and 0.1 rather than 10, 1, 0.1, and 0.01 µg.*

TABLE V Comparison of skin test reponses in dog No. 3 vaccinated with the CMR vaccine.

Hour after skin test	Skin test antigen	Response (mm erythema/induration)			
		10	1	0.1	0.01
0	PHASE I	0/49	0/48	0/52	0/57
4		36/68	4/61	0/55	1/62
24		24/58	0/54	0/54	0/59
48		0/51	0/50	0/49	0/57
72		0/58	0/53	0/51	0/56
96		0/57	0/53	0/49	0/51
120		0/56	0/53	0/52	0/54
0	PHASE II*	0/49	0/54	0/57	0/60
4		48/80	20/75	0/60	0/61
24		42/66	0/62	0/58	0/61
48		0/62	0/61	0/57	0/60
72		0/73	0/61	0/56	0/57
96		0/71	0/68	0/54	0/53
120		0/69	0/59	0/56	0/56
0	CMR	0/48	0/51	0/54	0/56
4		48/71	0/74	0/74	0/62
24		0/53	0/55	0/62	0/64
48		0/50	0/53	0/51	0/54
72		0/60	0/52	0/55	0/57
96		0/61	0/53	0/56	0/56
120		0/59	0/53	0/56	0/55

* *The skin test dose was 100, 10, 1, and 0.1 rather than 10, 1, 0.1, and 0.01 µg.*

TABLE VI Comparison of skin test reponses in dog No. 4 vaccinated with the CMR vaccine.

Hour after skin test	Skin test antigen	Response (mm erythema/induration)			
		10	1	0.1	0.01
0	PHASE I	0/63	0/66	0/76	0/101
4		72/91	24/86	0/91	0/100
24		6/72	0/79	0/79	0/96
48		ND**	ND	ND	ND
72		ND	ND	ND	ND
96		ND	ND	ND	ND
120		ND	ND	ND	ND
0	PHASE II*	0/57	0/69	0/89	0/107
4		18/82	0/71	0/88	0/97
24		15/76	0/71	0/84	0/97
48		ND	ND	ND	ND
72		ND	ND	ND	ND
96		ND	ND	ND	ND
120		ND	ND	ND	ND
0	CMR	0/55	0/64	0/87	0/100
4		72/80	2/78	0/90	0/104
24		30/74	0/75	0/97	0/130
48		ND	ND	ND	ND
72		ND	ND	ND	ND
96		ND	ND	ND	ND
120		ND	ND	ND	ND

* *The skin test dose was 100, 10, 1, and 0.1 rather than 10, 1, 0.1, and 0.01 µg.*
** *Not done. The dog died inadvertently.*

TABLE VII Comparison of skin test reponses in dogs No. 5 and 6 vaccinated with the WCII vaccine.

Hour after skin test	Skin test antigen	Response (mm erythema/induration)			
		100	10	1	0.1
Dog 5					
0	PHASE II	0/51	0/48	0/55	0/56
4		63/62	0/60	18/63	6/59
24		15/52	0/51	0/56	0/62
48		0/48	0/48	0/52	0/52
72		0/52	0/50	0/52	0/53
96		36/71	0/54	0/53	0/54
120		0/55	0/53	0/54	0/56
Dog 6					
0	PHASE II	0/54	0/54	0/59	0/61
4		4/60	0/58	0/58	0/58
24		0/66	0/59	0/59	0/59
48		0/64	0/58	0/58	0/60
72		0/91	0/62	0/54	0/57
96		130/130	56/130	30/105	0/63
120		0/130	0/130	0/124	0/59

DISCUSSION

The enzootic cycles of C. *burnetii* infection among wild and domestic animals suggest that the only practical method of controlling the spread of the microorganism to susceptible end hosts (humans) is through vaccination of secondary reservoir hosts. Safe, immunogenic and efficacious phase I *C. burnetii* vaccines have been developed for Q fever and coxiellosis. In previous studies, scientists have noted that dogs play the role of sentinel animals because they share the home and the surrounding environment with humans (18). Carnivorous pet animals such as cats and dogs are involved in the transmission of *C. burnetii* to humans. Although eradication of *C. burnetii* from the environment is not practical, vaccination of pet animals is a method of controlling this infection in animals and preventing the dissemination of the microorganism to humans. We have presented results which indicate that Q fever vaccines may be used to immunize dogs. The level of immunity induced by these Q fever vaccines may protect dogs against *C. burnetii* infection.

A comparison of phase I Q fever vaccines in dogs has revealed that WCI and CMR induced significant humoral and cellular immune responses to phase I and phase II *C. burnetii* (tables I to VI). WCII vaccine only induced humoral responses to phase II antigen (table I), and the animals exhibited cellular immune responses to phase I and phase II antigens (table II). Although the experimental groups were small, the CMR vaccine induced higher antigen-specific antibody levels than the WCI and WCII vaccines (table I), and primed lymphocytes responded just as well as WCI and WCII vaccinated animals (table II).

Granulomas are part of the natural pathology of Q fever and coxiellosis. The induction of dermal granulomas by Q fever antigens in humans and guinea pigs is a sensitive method to detect CMI and possible adverse vaccine reactions in previously sensitized humans (1) and animals (2). The dermal skin responses of dogs sensitized with WCI, WCII and CMR vaccines have been tested (tables III to VII). The time course of erythema and induration after skin test with *C. burnetii* antigens are suggestive of immunity and granuloma formation, respectively. The early (4 hours) erythema responses were obtained with all skin test antigens regardless of the sensitizing vaccine antigen. Late (96 h) erythema responses were obtained with all skin test antigens in animals sensitized with WCI or WCII. Only the CMR vaccine sensitized animals to respond with an early induration.

Important distinctions between skin test responses were noted in the animals sensitized with the WC vaccines and the CMR subunit vaccine (table VIII). Animals sensitized with WCI or WCII vaccines developed only late induration reactions to the skin test antigens. Neither the late erythema nor the late induration reactions were obtained after skin testing the dogs vaccinated with CMR. These skin test reactions are similar to those obtained by vaccinating and skin testing guinea pigs with CMR (2). The CMR has retained the same immunogenic potential for dogs as shown for mice and guinea pigs (19, 20, 22). The humoral and cellular immune response of dogs vaccinated with CMR suggests that the CMR determinants which protect mice and guinea pigs from lethal *C. burnetii* infection may also protect dogs.

TABLE VIII Comparison of skin test reactions after vaccinating with Q fever vaccines.

Vaccine	Skin test response	Skin test activity*		
		Phase I	Phase II	CMR
Phase I	Erythema			
	Early	yes	yes	yes
	Late	yes	yes	yes
	Induration			
	Early	no	no	no
	Late	yes	yes	yes
Phase II	Erythema			
	Early	NT**	yes	NT
	Late	NT	yes	NT
	Induration			
	Early	NT	no	NT
	Late	NT	yes	NT
CMR	Erythema			
	Early	yes	yes	yes
	Late	no	no	no
	Induration			
	Early	yes	yes	yes
	Late	no	no	no

** Any erythema was graded as yes. Induration with a ≥ 20 mm change was graded as yes.*
*** NT = Not tested.*

The severe adverse reactions noted for dogs in this study were not observed in mice, guinea pigs, sheep and goats in previous studies (19, 20, 22). Either the antigens or the FIA or the combinations induced adverse immune responses unique to dogs. FIA is known to stimulate macrophages, promote uptake of antigen, enhance antibody formation, and induce granuloma formation, but not to induce CMI (15). Phase I WC and CMR also possess adjuvant-like activities which enhance non-specific resistance to various pathogens and stimulates lymphokine production in mice (14). The phase I vaccines in FIA induced abscesses earlier (i.e., by day 19 to 24) than the phase II vaccine in FIA (i.e., by day 104). This suggests that the *C. burnetii* antigens may play a role in the induction of abscesses in dogs. Even though all three vaccines induced adverse reactions, the CMR vaccine did not induce the late skin test induration reactions characteristic of WCI vaccines that induce granulomas or sterile abs-

cesses in sensitive human recipients. CM extraction was shown to remove the determinants that cause granulomas in guinea pigs (2). Moreover, the reconstitution of CMR with the CM extracted lipids restores the granuloma inducing capability of the CMR to induce severe reactions in mice (20). Perhaps the CMR-oil-and-water emulsion restored this activity in dogs.

CONCLUSION

The Q fever vaccines tested in this study were immunogenic, inducing both humoral and cellular immune responses. The CMR vaccine induced greater humoral antigen-specific antibody responses than did the WCI and WCII vaccines. Cellular immunity was induced by all three vaccines as evidenced by *in vitro* lymphoproliferative assays and by *in vivo* skin testing. The CMR vaccine as skin test antigen did not induce the late induration responses which are characteristic of *C. burnetii* WCI and WCII vaccines. Although all three vaccines caused induration and sterile draining abscesses, the abscesses resolved within 30 days. These unacceptable adverse reactions could have been induced by FIA, the antigens or the combinations. There remain further study to determine the source of these adverse reactions and to evaluate the effectiveness of these vaccines against coxiellosis in dogs.

REFERENCES

1. ASCHER (M.S.), BERMAN (M.A.), RUPPANNER (R.). Initial clinical and immunologic evaluation of a new phase I Q fever vaccine and skin test in humans. *J. Infect. Dis.*, 1983, **148** : 214-222.

2. ASCHER (M.S.), WILLIAMS (J.C.), BERMAN (M.A.). Dermal granulomatous hypersensitivity in Q fever : comparative studies of the granulomatous potential of whole cells of *Coxiella burnetii* phase I and subfractions. *Infect. Immun.*, 1983, **42** : 887-889.

3. DERRICK (E.H.). "Q" fever a new fever entity : clinical features. diagnosis and laboratory investigation. *Med. J. Aust.*, 1937, **2** : 281-299.

4. FISET (P.), ORMSBEE (R.A.), SILBERMAN (R.), PEACOCK (M.), SPIELMAN (S.H.). A microagglutination technique for detection and measurement of rickettsial antibodies. *Acta Virol., (Prague, Eng. ed.)*, 1969, **13** : 60-66.

5. LANG (G.H.). Coxiellosis (Q fever) in animals. *In :* MARRIE (T.J.). Ed. Q Fever. Vol I. The Disease. Boca Raton, FL, CRC Press, 1990. P. 23-48.

6. LAUGHLIN (T.), WAAG (D.), WILLIAMS (J.), MARRIE (T.). Q fever : from deer to dog to man. *Lancet*, 1991, **337** : 676-677.

7. LUOTO (L.), CASEY (M.L.), PICKENS (E.G.). Q fever studies in Montana. Detection of asymptomatic infection among residents of infected dairy premises. *Am. J. Epidemiol.*, 1965, **81** : 356-369.

8. MARMION (B.P.), ORMSBEE (R.A.) KYRKOU (M.), WRIGHT (J.), WORSWICK (D.A.), IZZO (A.A.), ESTERMAN, (A.), FEERY (B.), SHAPIRO (R.A.). Vaccine prophylaxis of abattoir-associated Q fever : eight years experience in Australian abattoirs. *Epidemiol. Infect.*, **104** : 275-287.

9. MARRIE (T.J.), DURANT (H.), WILLIAMS (J.C.), MINTZ (E.), WAAG (D.M.). Exposure to parturient cats : a risk factor for acquisition of Q fever in maritime Canada. *J. Infect. Dis.*, 1988, **158** : 101-108.

10. MARRIE (T.J), SCHLECH (W.F.), WILLIAMS (J.C.), YATES (L.). Q fever pneumonia associated with exposure to wild rabbits. *Lancet*. 1986, **1** : 427-429.

11. MEIKLEJOHN (G.), REMER (L.G.), GRAVES (P.S.), HELMICK (C.). Cryptic epidemic of Q fever in a medical school. *J. Infect. Dis.*, 1981, **144** : 107-114.

12. ORMSBEE (R.A.), MARNION (B.P.). Prevention of *Coxiella burnetii* infection-vaccines and guidelines for those at risk. *In* : MARRIE (T.J.), ed. Q Fever. Vol. I. The Disease. Boca Raton, FL, CRC Press, 1990. P. 225-248.

13. SCHMEER (N.), MULLER (P.), LANGEL (J.), KRAUSS (H.), FROST (J.W.), WIEDA (J.). Q fever vaccines for animals. *Zbl. Bakt. Hyg. A*, 1987, **267** : 79-88.

14. WAAG (D.M.), KENDE (M.), DAHROW (T.A.), WOOD (O.L.), WILLIAMS (J.C.). Injection of inactivated phase I *Coxiella burnetii* increases non-specific resistance to infection and stimulates lymphokine production in mice. *Ann. N. Y. Acad. Sci.*, 1990, **590** : 203-214.

15. WAKSHAN (B.H.). Adjuvants and immune regulation by lymphoid cells. *In* : CHEDID (L.), MIESCHER (P.A.), MUELLER-EBERHARD (H.J.), Eds. Immunostimulation. Springer seminars in Immunopathology 2. Berlin, Heidelberg and New York, Springer Verlag, 1980. P. 4-33.

16. WAXMAN (F.J.), CLEMMONS (R.M.), HINRICHS (D.J). Progressive myelopathy in older german shepherd dogs. *J. Immunol.*, 1980, **124** : 1216-1222.

17. WELSH (H.H.), LENNETTE (E.H.), ABINANTI (F.R.), WINN (J.F.). Air-borne transmission of Q fever : the role of parturition in the generation of infective aerosols. *Ann. N. Y. Acad. Sci.*, 1958, **70** : 528-540.

18. WILLEBERG (P.), RUPPANNER (R.), BEHYMER (D.E.) HAGHIGHI (S.), KANEKO (J.J.), FRANTI (C.E.). Environmental exposure to *Coxiella burnetii:* a sero-epidemiologic survey among domestic animals. *Am. J. Epidemiol.*, 1980, **111** : 437-443.

19. WILLIAMS (J.C.), CANTRELL (J.L.) . Biological and immunological properties of *Coxiella burnetii* vaccines in C57BL/10ScN endotoxin-nonresponder mice. *Infect. Immun.*, 1982, **35** : 1091-1102.

20. WILLIAMS (J.C.), DAMROW (T.A.), WAAG (D.M.), AMANO (K.I.). Characterization of a phase I *Coxiella burnetii* chloroform-methanol residue vaccine that induces active immunity against Q fever in C57BL/10ScN mice. *Infect. Immun.*, 1986, **51** : 851-858.

21. WILLIAMS (J.C.), PEACOCK (M.G.), McCAUL (T.F.). Immunological and biological characterization of *Coxiella burnetii* phases I and II, separated from host components. *Infect. Immun.*, 1981, **32** : 840-851.

22. WILLIAMS (J.C.), PEACOCK (M.G.), WAAG (D.N.), KENT (G.), ENGLAND (M.J.), NELSON (G.), STEPHENSON (E.H.). Vaccines against coxiellosis and Q fever : Development of a chloroform: methanol residue subunit of phase I *Coxiella burnetii* for the immunization of animals. *Ann. N. Y. Acad. Sci.*, 1992, **653** : 88-111.

23. WILLIAMS (J.C.), SANCHEZ (V.). Q fever and coxiellosis. *In :* BERAN (G.), BENENSON (W.), TORTEN (A.S.), DREESEN (D.), RISTIC (M.), PIER (A.), SMITH (K.), KUNESH (J.), Eds. Handbook series on zoonoses. Boca Raton, FL, CRC Press. 1993 (in press).

J.C. Williams M.G. Peacock R.E. Race

WILLIAMS (J.C.), PEACOCK (M.G.), RACE (R.E.). Immunization of dogs with Q fever vaccines : comparison of phase I, phase II and phase I CMR *Coxiella burnetii* vaccines. *Revue Élev. Méd. vét. Pays trop.*, 1993, **46** (1-2) : 87-94

Q fever vaccines were tested in mixed breed dogs by vaccinating them with formalin-killed *Coxiella burnetii* whole cells (WC) in either phase I (WCI) or phase II (WCII), or the chloroform : methanol residue (CMR) subunit of phase I cells. Phase I vaccines mixed (1:1) with Freund's incomplete adjuvant (FIA) induced humoral immune responses to phases I and II antigens as measured by microagglutination assay. The CMR vaccine mixed (1:1) with FIA induced greater antigen-specific antibody levels to both phases I and II antigens than the corresponding WCI vaccine. The WCII vaccine induced antibody responses to only phase II antigens. The time course of erythema and induration after skin testing with *C. burnetii* antigens were suggestive of cell-mediated immunity (CMI). Although granulomas were observed with only WCI and WCII, none of the skin test antigens induced abscesses at the injection site. In contrast, axillary nodes draining the vaccine injection site developed sterile draining abscesses in all dogs by days 19 to 24 for the WCI and CMR, and day 104 for the WCII vaccines. The abscesses had resolved within 30 days after first appearance. Responses to Con A and PHA and recall antigens of lymphocytes from the blood, axillary and mesenteric nodes, and spleen at 222 days after vaccination were variable among dogs. Lymphocytes from various organs responded to one or more of the recall antigens and to both mitogens in the absence or presence of indomethacin. Although these Q fever vaccines induced humoral and CMI, either the antigens or FIA caused sterile draining abscesses. The skin testing results suggest that the CMR vaccine is a better alternative than the WC vaccines because of the lack of late granuloma formation by CMR. There remains further studies to determine the source of the adverse reactions and to evaluate the effectiveness of the vaccines against coxiellosis in dogs.

Key words : Dog - *Coxiella burnetii* - Q fever - Vaccine - Immunization - Immune response - Immunological techniques - Cell mediated immunity.

WILLIAMS (J.C.), PEACOCK (M.G.), RACE (R.E.). Inmunización en perros con vacunas de fiebre Q : comparación de vacunas de *Coxiella burnetii* en la fase I, fase II y del résiduo cloroformo/metanol (RCM) en la fase I. *Revue Élev. Méd. vét. Pays trop.*, 1993, **46** (1-2) : 87-94

Se probaron vacunas contra fiebre Q en perros de razas cruzadas, mediante vacunación con células completas de *Coxiella burnetii* (CC), muertas en formalina, tanto en la fase I como en la fase II, o con células de sub-unidades de residuos de cloroformo/metanol de la fase I. Mediante mediciones por ensayos de microaglutinación, se determinó que las vacunas fase I mezcladas (1:1) con adyuvante de Freund incompleto (AFI), indujeron respuestas inmunes humorales con antígenos de las fases I y II. La vacuna RCM mezclada con AFI (1:1) indujo niveles más elevados de antígenos específicos y anticuerpos, que la CCI correspondiente, tanto con antígenos de la fase I como de la II. La vacuna CCII indujo respuestas de anticuerpos solamente con antígenos de la fase II. La duración del eritema y la induración en piel después de la prueba con antígenos de *C. burnetii,* sugieren la presencia de inmunidad celular. A pesar de que se observaron granulomas con la CCI y la CCII, ninguno de los tests antigénicos en piel indujeron abscesos en el sitio de inyección. Sin embargo, los nódulos axilares que drenaban el sitio de inyección, sí desarrollaron abscesos estériles de drenaje en todos los perros, entre el día 19 y 24 para CCI y RCM y al día 104 para las vacunas CCII. Los abscesos desaparecieron aproximadamente 30 días después de la aparición. Doscientos veintidós días post vacunación, las respuestas a Con A y PHA y la tasa de antígenos linfocitarios de llamado en la sangre, los nódulos axilares y mesentéricos y del bazo, fueron variables en todos los perros. En varios órganos, los linfocitos respondieron a uno o a varios antígenos de llamado y a ambos mitógenos, tanto en presencia como en ausencia de indometacina. A pesar de que estas vacunas contra fiebre Q inducen inmunidad humoral y celular, se observan abscesos estériles de drenaje, producidos ya sea por el antígeno o por el AFI. Los resultados de los tests en piel sugieren que la vacuna RCM es una mejor alternativa que las vacunas WC, debido a la ausencia de formacion tardía de granuloma por parte del RCM. Se recomienda la realización de estudios posteriores, con el fin de determinar el origen de las reacciones secundarias y de evaluar la eficiencia de la vacuna contra la coxielosis en perros.

Palabras claves : Perro - *Coxiella burnetii* - Fiebre Q - Vacuna - Inmunización - Respuesta inmunitaria - Técnica inmunológica - Inmunidad celular.