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Vaccine prophylaxis of abattoir-associated Q fever: eight years' experience in Australian abattoirs

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SUMMARY

During the period 1981–8 a clinical trial of a Q fever vaccine (Q-vax; Commonwealth Serum Laboratories, Melbourne) has been conducted in abattoir workers and other at-risk groups in South Australia. Volunteers in four abattoirs and visitors to the abattoirs were given one subcutaneous dose of 30 μg of a formalin-inactivated, highly-purified *Coxiella burnetii* cells, Henzerling strain, Phase 1 antigenic state, in a volume of 0·5 ml.

During the period, over 4000 subjects have been vaccinated and the programme continues in the abattoirs and related groups. 'Common' reactions to the vaccine comprised tenderness and erythema, rarely oedema at the inoculation site and sometimes transient headache. Two more serious 'uncommon' reactions, immune abscess at the inoculation site, were observed in two subjects, and two others developed small subcutaneous lumps which gradually dispersed without intervention.

Protective efficacy of the vaccine appeared to be absolute and to last for 5 years at least. Eight Q fever cases were observed in vaccinees, but all were in persons vaccinated during the incubation period of a natural attack of Q fever before vaccine-induced immunity had had time ($\geqslant$ 13 days after vaccination) to develop. On the other hand, 97 Q fever cases were detected in persons working in, or visiting the same abattoir environments.

Assays for antibody and cellular immunity showed an 80–82% seroconversion after vaccination, mostly IgM antibody to Phase 2 antigen, in the 3 months after vaccination. This fell to about 60%, mostly IgG antibody to Phase 1 antigen, after 20 months. On the other hand, 85–95% of vaccinees developed markers of cell mediated immunity as judged by lymphoproliferative responses with *C. burnetii* antigens; these rates remained elevated for at least 5 years.

The Q fever vaccine, unlike other killed rickettsial vaccines, has the property of

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stimulating long-lasting T lymphocyte memory and this may account for its unusual protective efficacy as a killed vaccine.

INTRODUCTION

In 1979–80 there was a sharp increase in the prevalence of Q fever in South Australian abattoirs related mainly to the introduction and slaughtering of feral goats, although cattle and sheep continued as a source of infection.

There was no realistic way of protecting abattoir workers against the airborne infection with *Coxiella burnetii* other than by vaccination. Consequently, in mid 1981, clinical trials of an inactivated, whole cell Q fever vaccine (Q-vax) made by the Commonwealth Serum Laboratories (CSL), Melbourne, were started in two South Australian abattoirs (SCR and DJ) [1], and in March 1989, Q-vax was approved by the Department of Community Services and Health, Canberra, for marketing in Australia.

Previous experience [2–6] with vaccination of laboratory workers handling *C. burnetii* showed that a few vaccinees develop chronic induration or a sterile abscess at the inoculation site. By the late 1950s it was established [3, 6] that this troublesome complication was a consequence of pre-existing immunity and could be avoided by pretesting subjects for Q fever antibody and for skin test reactivity [7]; clear positives were excluded from vaccination.

Nevertheless, as some 25–50% of abattoir workers have immune markers [1] (see also Table 1) after previous clinical or subclinical infection, and also because at the start of the trial a low level of Q fever antibody or a minimal or equivocal skin test reaction was of uncertain predictive value, either for vaccine reactions, or for immunity to infection, a first priority of the SA trials was to establish whether or not the vaccine could be given without hazard to the abattoir workers. Secondary objectives of the trial at that time were to calibrate the immune response to vaccine, to obtain evidence of vaccine-induced protection, and to monitor for vaccine-enhanced disease.

A report [1] on the first 18 months of the SA trial established that the CSL Q fever vaccine had not produced severe reactions at the vaccination site. Serological conversion rates after vaccination, as measured by complement fixation or immunofluorescence tests, were 54% in subjects at one abattoir, and 64% in the other. Response rates were higher, 76–82%, in vaccinees with weak or border-line positive serological reactions before inoculation, suggesting previous exposure.

Finally, overall during 1981–2, there were no Q fever cases amongst 924 vaccinees at the two abattoirs, except for four subjects who had been vaccinated during the incubation period (about 20 days) of a natural attack of Q fever. On the other hand, there were 34 cases of Q fever among 1349 unvaccinated persons in the same environments. Differences were statistically significant ($P < 0{\cdot}05$) when considered either as simple attack rates or differences in incidence rates per 1000 exposure months [8].

Vaccine-induced immunity appeared to take about 13 days to develop and then to be complete, and not to be accompanied by modified or severe (i.e. vaccine-enhanced) Q fever as immunity waned.

An open trial design had been chosen to answer the questions above. First, for

ethical reasons, as several vaccination-challenge trials [6, 9, 10] in volunteers had shown a similar vaccine to be highly protective. And, second, because major elements in the success or failure of a vaccine programme are the perceptions of risk and of benefit among the participants; in 1981 the abattoir workforce would have had difficulty with a proposition that half of their co-workers eligible for vaccination should be denied protection so as to act as a control group.

Once the first 18 months of the open trial had been completed without significant reactogenicity and without apparent vaccine failures, it was possible to extend the trial to increase experience of vaccine safety and of the nature and duration of the immune response; also to meet the tenets of the conventional wisdom about bias factors in open trials by arranging a limited, 'blind', placebo-controlled trial, so designed to cost only a few cases of Q fever in the placebo group before it could be concluded and the latter revaccinated with Q fever vaccine. This trial in sequential analysis format [11] was done in Queensland, a state with the highest prevalence of Q fever in Australia (see accompanying paper by Shapiro and co-workers). As in the open trial in South Australa, Q-vax proved to be completely protective.

The present, final report draws together 8 years of experience with Q-vax, eventually involving over 4000 vaccinees working in or visiting four abattoirs in South Australia and over 500 in Queensland and other states.

An analysis is made of bias factors in the assessment of vaccine efficacy in South Australia by standard epidemiological techniques. Also, the results of extensive studies of humoral and cell-mediated responses to the vaccine and other strains of *C. burnetii* are summarized.

TRIAL ORGANISATION AND METHODS

Tests for existing immunity before vaccination

Subjects were serotested and skin tested [1, 12]. After 1984, in anticipation of a wider use of vaccine, the pretesting procedure was simplified by testing for CF antibody to Phase 1 and Phase 2 antigens at dilutions of 1 in 2·5, 5, 10 and 20, and by skin testing at the time of taking the blood sample. Seven days later, the skin test was read, and the serological results had become available; negative subjects could then be vaccinated. Under this modified scheme, further tests of sera by IF were sometimes necessary to determine, for example, the significance of CF antibody at a dilution of 1 in 2·5 in the presence of a negative skin test.

Vaccination procedure

Subjects without clear-cut immune markers were vaccinated [1]. The vaccine was prepared at CSL by the Ormsbee method [13, 14] from the Henzerling strain of *C. burnetii* maintained in the Phase 1 antigenic state. The seed strain used had been shown to be free of contaminating avian viruses or mycoplasmas, and was propagated in specific pathogen-free chick embryos. Formalin-inactivation of the infectivity of *C. burnetii* took place throughout the process of harvesting and purification of the coxiellas from the chick embryo yolk sacs. Tests for residual viable organisms were done on 100 dose lots of the purified, formalin-treated vaccine, and involved four serial passages in chick embryo yolk sac with staining

for organisms in yolk sac smears, following a protocol specified by the National Biological Standards Laboratory, Canberra. In addition, one lot of vaccine concentrate was inoculated IP in mice, the spleens harvested after 14 days, homogenized, and suspensions passed serially three times in 6-day-old CE yolk sacs. Yolk sac suspensions were subinoculated into pre-bled guinea-pigs tested for CF and IF Q fever antibody at 3 and 6 weeks after inoculation.

The third yolk sac passage suspension was also inoculated into mice which were subsequently tested for antibody. Smears were made from all yolk sacs and examined for coxiellas by IF with a high titre serum against Phase 1 antigen. None of these procedures revealed viable *C. burnetii*.

RESULTS

Studies in South Australia

During the period from July 1981 to December 1988, over 6000 subjects in South Australia have been enrolled in the vaccine programme and pretested, and over 4000 have been vaccinated. The majority were workers at four abattoirs (SCR, DJ, METNOR and CDMB), but vaccine was also offered via the Vocational Health Resources Clinic (VHRC), Mile End, Adelaide, to a heterogenous group of persons (e.g. insurance assessors, mechanical and electrical tradesmen, Telecom technicians, catering school students, biological research workers), who, for various reasons, had to visit the abattoirs and were therefore at risk.

For some assessments of the vaccine, e.g. reactogenicity, use is made of data from the whole period 1981–8. For detailed statistical analysis of protective efficacy, the period from mid 1981 to the end of 1986 is chosen and covers 5739 enrolled subjects, with 3309 vaccinated at the four abattoirs, and 235 subjects enrolled at VHRC with 212 vaccinated (Table 1).

Local and general reactions to vaccine in South Australian studies

As indicated [1] the common or trivial reactions to vaccination in 464 vaccinees were tenderness at the inoculation site (48%, lasting 1–3 days), erythema at the inoculation site (33%, lasting 1–3 days), transient headache (9%, lasting 1 day). In this group of 464 subjects, induration or oedema at the vaccination site was rare (under 1%). Fever was also uncommon (0·2%).

The occurrence of uncommon, persistent and severe reactions at the vaccination site, lasting 7 days or more, has been monitored throughout the period. The medical records of 820 (95%) of 869 vaccinees, 1981–6, at SCR were searched systematically for entries, sickness certificates, or compensation claims relating to persistent reactions at the inoculation site; none were found (at the same time investigations were made for possible missed Q fever cases). A questionnaire was also issued to the 869 vaccinees and was answered by 28%. One subject recorded a small, mobile lump at the site of inoculation which lasted for about 2 months before it disappeared. Three hundred and twenty-five vaccinees at SCR and DJ were re-examined 6–12 months after inoculation for induration at the vaccination site; none was found.

In total, 2682 (75%) of the records of 3532 subjects vaccinated 1981–6 at the four abattoirs or at the VHRC were reviewed for chronic reactions, either from

their medical records or from the survey forms completed for each subject; all were negative. The medical officers and the nursing staff at the abattoirs and VHRC were also questioned but no persistent reactions had been reported to them from the group of 3532 subjects.

The only significant chronic reaction in the whole series was an abscess at the vaccination site in a Master Butcher with 30 years' service in the meat industry, and resembled those described previously [3]. He had been vaccinated at SCR, but was not an employee of that organization, so is not mentioned in the search of records just described. His prevaccination skin test was read as negative, although there was some slight induration when it was reviewed at the time, 7 days after vaccination, when an abscess developed at the inoculation site. His prevaccination, Q fever serum antibody titres were CF 2·5, IF 10, and did not increase during the course of development of the abscess and its resolution.

Exudate from the abscess did not show significant numbers of pyogenic bacteria, however the content of *C. burnetii* antibody, proportional to serum albumin, was much higher than in the blood. In addition, he had a vigorous lymphoproliferative response when his peripheral blood mononuclear cells were stimulated with *C. burnetii* antigens. The draining track from the abscess was excised and the wound sutured; the abscess then healed.

There were no examples of persistent, general or systemic reactions clearly attributable to vaccination and lasting more than 7 days.

Reactions to vaccine in the Queensland trial

Of the 200 vaccinees in the trial, 98 were given Q fever vaccine (Q-vax, CSL) and 102 given influenza vaccine (Flu-vax, CSL).

There were no reactions to the influenza vaccine but nine (9·2%) of those given Q-vax had 'common' reactions as defined and described above. One subject had a tender, soft subcutaneous swelling (1·5 cm diam) superficial to the insertion of the left deltoid muscle. It disappeared after 3 months without intervention.

Q fever in vaccinated or unvaccinated persons working in or having other forms of contract with the four study abattoirs

Clinical trials of Q fever vaccine began at SCR (Gepps Cross and Port Lincoln) and at DJ (outside Adelaide) in mid 1981. SCR Port Lincoln closed in 1983; in September 1986, DJ ceased to slaughter goats and reverted to the slaughtering of pigs. The METNOR and CDMB abattoirs, at Noarlunga and Murray Bridge respectively, joined the trials in October 1985 and June 1986. From February 1986, vaccine was offered at VHRC, Adelaide to other groups with direct or indirect contact with the four abattoirs.

Table 1 summarizes the number of vaccinated subjects working in, or otherwise connected with, the four abattoirs, subgrouped in terms of their consistency of exposure to the abattoir environments. It also shows the proportion in each subgroup with immune markers on enrolment, together with the number of Q fever cases in vaccinated and unvaccinated subjects in the subgroup.

Abattoir workers involved in the main activity of killing and dressing animal carcasses, and in disposing of offal and other byproducts, together with the Meat Inspectors, had the highest rates of immune markers on enrolment into the

Table 1. *Numbers of vaccinated and unvaccinated subjects and Q fever cases in groups working in or connected with four trial abattoirs in South Australia during the period 1981–6*

Employee category	Subgroup	Proportion (%) with immune markers on enrolment	Vaccine status	No. in subgroup	Q fever cases
Abattoir workers, Meat Inspectors	A	24–47	Vaccinated	2716	3*
			Unvaccinated	2012	52
Supporting firms on site	B	12	Vaccinated	269	3*
			Unvaccinated	(≥ 140)	24
Regular visits for mechanical or electrical servicing	C	13	Vaccinated	23	1*
			Unvaccinated	(≥ 7)	2
Sporadic visits or contact with material from abattoir	D	9	Vaccinated	524	0
			Unvaccinated	(≥ 48)	19
			Total subjects	5739	104

* Vaccinated during incubation period of a natural infection with Q fever.
( ), Number recorded and tested. This represents the lower limit of those exposed: see text.

programme (subgroup A, Table 1). In this subgroup, there were three cases of Q fever amongst 2716 vaccinees, all in individuals vaccinated during the incubation period of a natural attack. By contrast in the same subgroup, there were 52 cases of Q fever in 2012 unvaccinated subjects.

Similar differences in the prevalence of Q fever cases in vaccinated and unvaccinated subjects were observed in the employees (group B) of the supporting firms on the abattoir sites (e.g. animal and meat transporters, hide and pelt processors, animal fertilizer processors), or in those (groups C, D) visiting to service equipment or other purposes. In total, there were four Q fever cases in 816 vaccinees in categories B, C and D. All were in subjects vaccinated during the incubation period of a natural infection. In contrast, there were 45 Q fever cases in unvaccinated subjects in the same categories. For obvious reasons, however, the total numbers at risk in the latter unvaccinated categories was uncertain.

During the period from the end of 1986 to the end of 1988, a further 764 subjects have been vaccinated at the three abattoirs remaining in the programme. One more case of Q fever, – the 8th, has been observed in a vaccinated subject, who developed the disease 1 day after vaccination (i.e. once again, during the incubation period of a natural attack). No other cases have been observed in vaccinees to the date of this report (3 August 89).

Detailed analysis of Q fever at the SCR abattoir site

A detailed analysis of the prevalence of Q fever is given for the SCR abattoir at Gepps Cross, Adelaide, the largest of the four in the study. Figure 1 shows the prevalence of Q fever cases in all employees on the site by years since 1978; it also includes cases among visitors to the site or ones originating from animal products from the site.

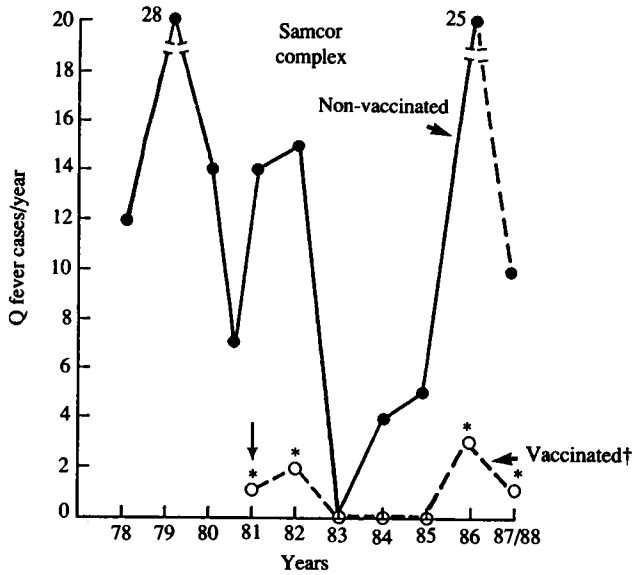

Fig. 1. Yearly prevalences, 1978–8, of Q fever cases in all employees on and visitors to the Samcor site or complex at Gepps Cross, Adelaide. Cases in vaccinated and unvaccinated are shown separately. *, Vaccinated in incubation period; †, total of 1922 vaccinated.

A fluctuating but significant prevalence of Q fever, averaging 14 cases per year, was observed during the period 1978–81. After the start of the vaccine programme in 1981, the general prevalence of Q fever cases continued, but reached a low level in 1983, a trend observed in other abattoirs and not attributed to the vaccine. This was followed by a sharp increase of cases during 1985–8. On the site generally, during the period 1981–8, there was a cumulative total of 1922 vaccinees. There were seven cases of Q fever among these vaccinees, all in persons who had been vaccinated during the incubation period of a natural attack. As will be seen from Fig. 1, there were no Q fever cases in subjects who had had time to develop immunity after vaccination, even in the face of a sharply increasing prevalence of Q fever during the years 1985–8.

Q fever amongst SCR employees

During the period mid 1981 to the end of 1986 chosen for detailed analyses, survey records were maintained on all 1562 employees of the SCR abattoir (i.e. those working on animal killing and other processing lines), as distinct from employees of the 15 or more firms providing a supporting role on the site. Of the total of 1562 subjects, 1338 were enrolled in the vaccine programme, and of the latter, 869 were vaccinated and 469 were not vaccinated, either because they had immune markers or because they did not take up the offer of vaccination (Table 2 and subgroups A and B in Table 2). The remaining 224 persons in the firm decided not to participate in the scheme (subgroup C, Table 2).

Analysis of the sex distribution in the three subgroups showed that the enrolled/vaccinated group contained proportionately more women than the other two groups ($P < 0{\cdot}0001$) and were younger (mean age for the vaccinated was

Table 2. *Q fever cases during the period 1981–6 among SCR employees in relation to pre-existing immunity and vaccination state*

Nature of subgroup	*N*	Proportion (%) with immune markers	Q fever cases	Exposure month (total)	Incidence rate per 1000 exposure months	
A						
Enrolled, Vaccinated	869	25	2*	20798	0·1 (a)†	
B						
Enrolled, Unvaccinated	469	87	9	18064	0·5 (b)†	
C						
Not enrolled, Unvaccinated	224	~40‡	11	7306	1·51	0·82(c)†
	(1338)		(0)	(6120)	0	

* Vaccinated during the incubation period of a natural attack of Q fever.

† Incidence ratios a/b = 1:5, $P = 0{\cdot}009$; a/c = 1:8·2, $P = 0{\cdot}0004$.

‡ Proportion for total sample of enrolled subjects is assumed to apply to the unenrolled subjects.

( ) Exposure months allocated to subgroup C from subgroups A and B and representing exposure *before* enrolment.

29·2 years (S.D. ± 12·9) and 38·0 years (S.D. ± 13·6) for the unvaccinated ($P < 0{\cdot}0001$)). Finally, the average length of service in the abattoir for each of the three groups was (*a*) enrolled and vaccinated, 1·98 years (S.D. ± 1·97); (*b*) enrolled but not vaccinated, 2·85 years (S.D. ± 2·08), and (*c*) not enrolled in the vaccine programme, 2·28 years (S.D. ± 2·05) ($P < 0{\cdot}0001$).

These differences in age, sex and length of service in the vaccinated and unvaccinated groups would bias *against* the observation of a protective effect of the Q fever vaccine if, in reality, it was of low or no activity. This is because it is precisely the younger age group newly recruited to the workforce, predominantly in the vaccinated group that, when unprotected, experiences the highest incidence of Q fever. Thus, preliminary studies before the vaccine programme started showed that during the first 5 years of service in the abattoir, workers rapidly acquire antibody to Phase 2 antigen; rates eventually reach 50–60% in those who have served 25 or more years. The proportion of workers with a positive skin test also increases markedly during this early period. Finally, an analysis of 113 cases of past Q fever cases showed that 66% of the total became ill with clinical Q fever within 10 years of starting work, and 42 (37%) within 5 years.

The observed differences in the age, sex and length of service between the enrolled/vaccinated, and the enrolled/unvaccinated groups, are very largely due to the selection of persons without immune markers for vaccination. From Table 3, it will be seen that of 869 vaccinated subjects, 179 (21%) had immune markers as compared with 408 (87%) of the 469 enrolled but unvaccinated individuals; the former had been vaccinated at an early stage of the programme when the significance of various combinations of weak antibody or skin test reactions was unclear.

Table 3 also includes subtotals for the exposure months in each of the four

Table 3. *Summary of the distribution of immune markers found on enrolment of a group of 1338 SCR employees in the vaccine programme. The numbers vaccinated in various categories are given, along with the exposure months in each category*

Immune markers on enrolment in the vaccine programme	Subjects enrolled and subsequently vaccinated			Subjects enrolled but not subsequently vaccinated			Totals
	No.	Exposure months (subtotal)	Cases of Q fever	No.	Exposure months (subtotal)	Cases of Q fever	
Positive*	179	6364	0	408	17254	2	587
Negative†	690	14434	2‡	61	810	7	751
Totals	869	20798	2	469	18064	9	1338

* Group contains those antibody positive/skin test positive, antibody positive/skin test not done, and antibody negative/skin test positive.

† Group contains those CF negative at $< 2{\cdot}5$ with a skin test considered negative by the physician and '0' mm diameter reaction.

‡ Vaccinated in the incubation period of a natural infection.

subgroups. These estimates of the duration of exposure are critical, given short length of service and the rapid turnover of abattoir employees; also the differing lengths of service of vaccinated and unvaccinated. Exposure months are calculated from the time of enrolment to the time of termination of employment, or to the end of 1986, as appropriate. Periods of exposure *before* enrolment in the group eventually vaccinated, or eventually enrolled but not vaccinated, have been allocated to the third group of 'not enrolled/unvaccinated' persons (subgroup C, Table 2).

Similar, although less exact, measurements of the exposure of the three groups, enrolled/vaccinated, enrolled/unvaccinated and not enrolled/unvaccinated, may be obtained by analysis of the retention in employment up to 1986 of each cohort of workers taken into employment in each of the years from 1981 to 1986 (and later enrolled in the vaccine programme). In brief, 35% of the enrolled/vaccinated joining the workforce in 1981–2 were still present in 1986, as against 45% of the enrolled/unvaccinated. However, only 20% of the unenrolled/unvaccinated joined in 1981 were present in 1986, but significantly, despite the rapid removal from exposure, this unprotected group had the highest incidence of Q fever cases (Table 2).

Two other conclusions may be drawn from the data in Table 3. Among the 408 enrolled/unvaccinated with immune markers, there were two Q fever cases; these had low antibody titres probably related to the developing Q fever illness. More importantly, the group of 690 subjects without immune markers, subsequently vaccinated, experienced two Q fever cases – an attack rate of 0·29%. The 61 unvaccinated subjects, also without immune markers, had seven Q fever cases, an attack rate of 11·5% (Fisher's exact test, $P < 0{\cdot}0001$). When analysed in terms of case-rates per 1000 exposure months, the 690 vaccinated experienced a case rate of 0·139 per 1000 exposure months as against a rate of 8·64 in the 61 unvaccinated – an incidence ratio of 1:63 ($P < 0{\cdot}0001$ by the method [8] for the significance of differences in incidence ratios).

This striking difference between the experience of vaccinated and unvaccinated is further reinforced by noting that the two Q fever cases in the group of 690 initially non-immune vaccinees were in fact vaccinated during the incubation period of a natural attack of Q fever. They were, nevertheless, left in the vaccinated group in order to bias the analysis *against* the effect we are seeking to demonstrate – namely, that the Q fever vaccine is protective. In fact, it appears that the vaccine is 100% protective if sufficient time elapses after vaccination for immunity to develop before exposure to natural infection, and from the cohort-retention study, immunity appears to last for at least 5 years.

Table 2 brings together the experience of all three subgroups at SCR, including that of the subgroup of 224 individuals who were not enrolled in the programme, about 40% of whom were estimated to have immune markers. This subgroup had the highest case incidence rate/1000 exposure months of 1·51. The row in Table 2 for this subgroup, C, has been credited with 6120 exposure months, representing the pre-enrolment exposure experience of subgroups A and B. (There were no Q fever cases matching these exposure periods because one criterion for enrolment was absence of previous clinical Q fever.) Once again the addition to the total of exposure months in subgroup C would diminish the case incidence rate and bias *against* a demonstration of vaccine efficacy. Despite this, comparison of the case incidence ratios for subgroups A, B and C shows a clear advantage for the enrolled/vaccinated.

Q fever in subjects vaccinated during the incubation period

The Q fever cases in vaccinees, eight in total from the four abattoirs during the entire period 1981 to 1988, are of particular importance in assessing the protective effects of the vaccine. The intervals between vaccination and onset of illness in the eight cases were 1, 1, 5, 7, 9, 11, 11 and 13 days respectively. The incubation period of naturally acquired Q fever is between 15 and 25 days.

Figure 2 shows the cumulative totals of Q fever cases in the enrolled-vaccinated in relation to the time between onset of illness and vaccination and, in the enrolled/unvaccinated, between onset of illness and enrolment. In the vaccinated, on average, about 7 days elapses between enrolment and vaccination.

It will be seen that the two curves for the cumulative totals of Q fever cases rise steadily during the first 10–15 days after vaccination or enrolment. However, the curve for the vaccinated reaches a plateau with no more cases after 13 days from vaccination whereas that for the unvaccinated continues to climb during the subsequent days and months. Two conclusions may be drawn. First, vaccinees are actually exposed to Q fever, i.e. there is no 'protective' effect simply from a prudent decision to be vaccinated linked to prudent work practices which avoid exposure to infection. Second, if the Q fever vaccine were valueless, it might be expected that the curves of total cases in the two groups would continue to rise in parallel as exposures occur at random with the arrival of infected animals and the vagaries of airborne infection. The absence of cases after 13 days from vaccination fits well with the observation (below) that cell-mediated immune responses are first demonstrable 10–13 days after vaccination.

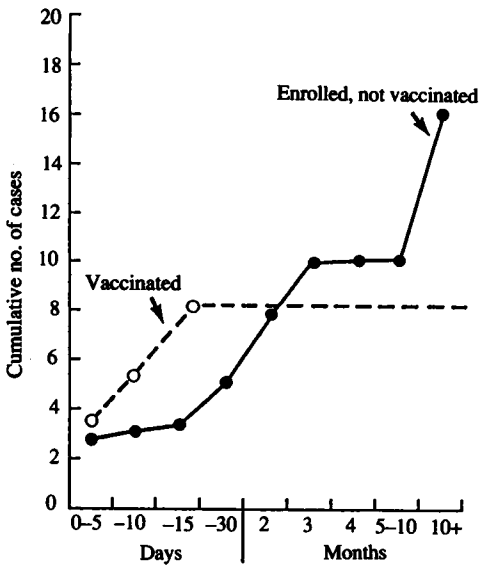

Fig. 2. Cumulative totals of Q fever cases in (*a*) vaccinees in relation to time since vaccination, and (*b*) enrolled but unvaccinated subjects in relation to time from enrolment. The usual interval between enrolment and vaccination is 7–10 days.

Immune response to vaccine

In view of the low antibody responses after vaccination previously reported [1, 12], a group of 50 subjects, seronegative and skin test negative before vaccination, was examined at various intervals from 3–60 months after vaccination, sera being tested by CF, IF and a solid-phase, competition RIA measuring antibody in any Ig class. Phase 1 and 2 antigens were used and IgM and IgG subclasses were checked in the IF assay (Worswick and colleagues, unpublished).

These measurements (Table 4) showed an 80–82% seroconversion rate between 3 and 19 months when results of all assays were combined. This early antibody response was predominantly in the IgM subclass. After 20 months, lower rates of antibody, 55–65%, were observed, mainly in the IgG class.

The frequency, and in particular the persistence of these antibody responses, contrasts with lymphoproliferative response (lymphocyte stimulation index; LSI) to *C. burnetii* Phase 1 and 2 antigens obtained with peripheral blood mononuclear cells (PBMC) obtained from vaccinees [15]. Eighty-seven to 95% of subjects had positive LSI 1 month after vaccination, and over 95% of vaccinees among abattoir workers had positive LSI 5 years after vaccination. Fractionation experiments found that the reactive cells were predominantly in the T lymphocyte fraction of the PBMC and the proliferative responses could be augmented by IL-2 treatment after antigen stimulation.

T lymphocyte reactivity was observed not only with the Henzerling Phase 1 strain of *C. burnetii*, the antigen in the vaccine, but also with the Nine Mile Phase 2 and Priscilla Phase 1 strains (Izzo, Marmion, Hackstadt, unpublished). As some abattoir infections arise from goats, the development of lymphocyte sensitization

Table 4. *Rates (%) of antibody detected and distribution by immunoglobulin class, in groups of vaccinees, serologically and skin test negative before inoculation, a few weeks to 3 months, and at 20–60 months after inoculation*

Time interval from vaccination (months)	Total no.	Antibody to *C. burnetii* antigen detected by: Immunofluorescence: IgM: Ph. 1	IgM: Ph. 2	IgG: Ph. 1	IgG: Ph. 2	All tests CF, IF, RIA
0·6–3	26	11	17	4	6	22
	(100)	(42)	(65)	(15)	(23)	(84)
20–60	47	4	9	21	6	30
	(100)	(8)	(19)	(45)	(13)	(64)

( ), percent of total (100%) in subgroup.

and memory to the Priscilla strain (isolated from goat placenta and representative of 'endocarditis' isolates [15, 16, 17] after vaccination with the Henzerling strain Q fever vaccine is significant for protection and in line with the protective effects observed in abattoir workers and cross-protection experiments in experimental animals [18].

DISCUSSION

The formalin-activated whole cell *C. burnetii* vaccine ('Q vax') appears to provide complete and long lasting protection. Despite the impressive record of whole cell vaccines, various other vaccine formulations have been explored in the hope of developing less reactogenic vaccines; these include extracted complexes of LPS and protein ('Chemovax'), chloroform-methanol extracted organisms and living attenuated strains of *C. burnetii*. The efficacy and reactogenicity of these preparations is reviewed in detail by Ormsbee and Marmion [19]. In brief, extracts and extracted organisms have protective efficacy but are not as immunogenic as the killed whole cell preparations. Living attenuated vaccines (e.g. the Russian M44 strain) have proved to be reactogenic and are open to the theoretical objection that the organism might be excreted in the placenta or colonize heart valves or valve protheses. We are not aware however that the latter complication was in fact detected in the Russian trials of living vaccine; in any event the special properties now attributed to endocarditis strains [16–18] might not be present in the living attenuated vaccine strains.

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