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TITLE

LIPOSOME ENCAPSULATED ANTIGENS AS VACCINES FOR BRUCELLA ABORTUS AND FRANCISELLA TULARENSIS

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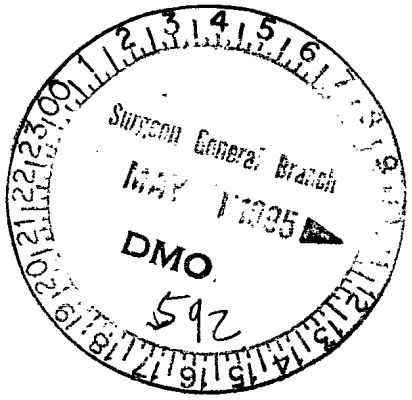

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FINAL REPORT:

TASK 0518B-11: DMO- 007

Liposome Encapsulated Antigens as Vaccines for *Brucella abortus* and *Francisella tularensis*

John W. Cherwonogrodzky
(scientific authority)

Department of National Defence, Defence Research Establishment Suffield, Box 4000, Medicine Hat, Alberta, Canada. T1A 8K6

ABSTRACT

From 30 August 1991 to 30 August 1995, DRES received a task from the Surgeon General's Office to develop a liposomal vaccine both to *Brucella abortus* and *Francisella tularensis*. Funding was $160,000 over these 4 years. Currently there is no vaccine for human use to the *Brucella* species. There is an effective attenuated vaccine to tularemia, *Francisella tularensis* live vaccine strain (LVS), but this bacterium can revert to a virulent form if cultural conditions are not followed as specified.

A novel purified polysaccharide vaccine has been produced for *B. abortus*. Liposomal encapsulation was found not to be required for its effectiveness. Protection did not appear to be due to an antibody response, and indeed may be counter-indicative. The vaccine was protective for mice and guinea pigs against *B. abortus* and for swine against *B. suis* (hence it appears to offer broad protection). Single injections of 100 ug (lesser amounts were not tested) were found to be 100% protective for 25 kg swine challenged with virulent field strains of *B. suis*. A year later these swine were still protected against brucellosis. Based on these results it was extrapolated that 300 ug might protect a 75 kg person. Initially 15,000 human dose equivalents was to be prepared for research and a potential emergency supply. The final preparation was enough for 150,000 human equivalent doses. Due to a lack of containment facilities, the vaccine could not be tested for protection against cross-reactive pathogens (e.g. *Francisella tularensis*).

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Background

Brucellosis is a debilitating disease that can cause abortions and weight loss in animals, "undulating" fevers, "night sweats", incapacitation and arthritis in humans. It is very hardy to environmental factors (about 1000-fold more resistant than other comparable bacteria), easily aerosolized and infectious through the pulmonary route, difficult to treat with antibiotics and often it persists as a life-long infection. It is an endemic disease to most countries, especially under-developed nations where it infects 5-10% of the livestock and people. *Brucella* was seriously considered as a BW weapon by the United States during WWII and Iraq did consider it under its (offensive/defensive) biological program. From 1982-1985 there was an unaccounted 100-fold increase in the brucellosis cases in Kuwait (Cherwonogrodzky and Di Ninno, 1994). The current view is that the *Brucella spp.* seldom kill their hosts. However over 100 years ago, Bruce, the discoverer of this bacterium, suspected that deaths were due to exceptionally virulent rare strains (Bruce, 1887). Our own studies with bacteriophage reversions appear to support the latter view (Cherwonogrodzky, unpublished results).

Currently there are no vaccines to protect people against brucellosis. In the past researchers have vaccinated people at high risk (e.g. veterinarians, abbatoir workers) with an attenuated vaccine strain, *B. abortus* S19, but this appears to be attenuated for cattle and is pathogenic for humans. There was a French vaccine that removed the toxic lipopolysaccharide (LPS) component with phenol, but the phenol insoluble residue gave a

high rate of reactogenicity (at least 53%) and led to hyper-sensitivity (vaccinates exposed to *Brucella* antigens were susceptible to anaphylactic shock). This latter vaccine has been discontinued and hence there are no human vaccines for brucellosis presently available.

At the start of the task to develop a vaccine against brucellosis, the view of the scientific community was exceptionally discouraging (identities are held as confidential). Below are the key points they raised:

1) *Brucella* was recognized over 100 years ago and for over a century researchers around the world have tried to raise a vaccine against brucellosis without success. Given the time, number of investigators and talent involved, the evidence was obvious that a vaccine could not be developed.
2) *Brucella* was a facultative parasite that could sequester inside tissues. Not only was it protected from antibiotics and vaccine-induced antibodies of humoral immunity, but it also had mechanisms for controlling its host phagocyte (i.e. it secretes thymidine and cyclic GMP which inactivate the host cell) and hence cellular immunity is ineffective.
3) Polysaccharides and bacterial glucans are very poor immunogens. The evidence is that these are the least likely candidates for vaccines.

Despite the views of world renowned *Brucella* experts and polysaccharide chemists, there were a few observations that gave indications that a vaccine was possible:

1) Protection does occur in the field. *Brucella* is only about 70% infectious (either to animals or people) which suggests that there is something occuring to protect the 30% which do not come down with brucellosis. Also, once a cow aborts due to *Brucella*, it has a natural immunity to this disease.

2) There was an unexplained but well accepted observation - although the O-polysaccharide or OPS (outer polysaccharide which gives a bacterium its serological identity) does not induce an immunological response, the immuno-dominant antigen of *Brucella* (about 80% of the antibodies are to this) is this same OPS when it forms part of the bacterial LPS.

3) I was the first one to determine that infected animals did produce antibodies which could precipitate OPS, and that animals vaccinated with the attenuated strain S19 had antibodies which could precipitate OPS only when it was part of LPS (Cherwonogrodzky et al., patent, 1986). It appeared to me that the OPS was somehow involved with immunity but that this immunity was inversely related to antibody activity. As investigators had found that LPS gave good antibody response but was not protective, and as no-one had tried OPS as a vaccine, there appeared to be an exceptional opportunity ignored by everyone else.

4) The other reason was chance occurrance. I had small amounts of OPS on hand for vaccine trials because of the new methods of its purification which I had developed (Cherwonogrodzky et al., 1990).

The other concept I had (which cannot at present be tested due to the lack of containment facilities) was the ability of one vaccine to protect against several diseases (several of which are BW candidates). Table I shows that several have similar OPS structures. Our own work gives evidence that *Francisella tularensis* may have a similar OPS to *Brucella* (Cherwonogrodzky et al., in preparation) and hence may a likely agent used as a challenge against the same OPS vaccine.

Table I: O-polysaccharide (OPS) of Cross-reactive Bacteria

Bacterium	O-polysaccharide on their LPS[a]
Brucella abortus	(1,2-linked) perosamine
Yersinia enterocolitica O:9	(1,2-linked) perosamine
Brucella melitensis	(1,3-linked) perosamine
Escherichia hermannii	(1,3-linked) perosamine
Brucella suis	(1,2/1,3-linked) perosamine
Vibrio cholerae	glycero-tetronic perosamine
Salmonella landau	glu,fu,acetyl-gal,acetyl-perosamine
Salmonella godesburg	glu,fu,acetyl-gal,acetyl-perosamine
Escherichia coli O157:H7	glu,fu,acetyl-gal,acetyl-perosamine
Pseudomonas maltophilia 555	rham,acetyl-gal,acetyl-perosamine
Francisella tularensis	dideoxy sugar-perosamine
Yersinia pestis	?-perosamine[b]

[a]perosamine, 4-formamido-4,6-dideoxy-D-mannose; glu, D-glucose; fu, L-fucose; gal, D-galactose, rham, D-rhamnose

[b]some strains of plague cross-react with *Brucella*, the mechanism is unknown (personal communication, Dr. M. Corbel, 1994)

Results

1) Mouse studies at DRES

Balb/c mice were immunized with purified OPS from *Brucella abortus* 1119-3, and initially the results were discouraging. As the experts had predicted, the IgG or IgM antibody titres (reflective of humoral immunity) were negligible with OPS, whether given as a single dose or as multiple (3) doses. The antibody titres were more pronounced when LPS was given as the antigen. The antibody titres could be enhanced when these antigens were liposomal encapsulated, but again the titres were still low for OPS (see Figures 1-8).

When these mice were challenged with a virulent strain of *B. abortus* 2308, however, OPS did appear to protect the mice from infection. Indeed, the poorer the antibody response to a given antigen, the better appeared to be the protection (Table II).

Table II: Balb/c Mice Vaccinated and then Challenged with *B. abortus* 2308

ANTIGEN	ANTIBODY titre[1] IGg	IgM	SPLEEN COUNT (log_{10}CFU)[2]	PROTECTION (no./total)	(%)
None	<6.6	<6.6	6.1, 6.5, 6.5, 6.6 (average 6.4)	0/4	0
LPS -1 μg	8.6	7.6	3.7, 4.8, 4.8, 5.8	1/4	25
-100 μg	11.6	8.6	2.6, 2.6, 3.7, 6.0	3/4	75
LIP-LPS-1 μg	11.6	7.6	3.0, 3.2, 4.6, 5.9	2/4	50
-100 μg	13.6	10.6	4.3, 4.7, 4.7, 6.3	1/4	25
OPS -1 μg	7.6	<6.6	3.7, 5.0, 5.5, 5.6	1/4	25
-100 μg	8.6	6.6	0, 0, 3.2, 3.4	4/4	100
LIP-OPS-1 μg	7.6	<6.6	0, 0, 3.3, 3.8	4/4	100
-100 μg	11.6	7.6	3.3, 3.4, 3.7, 4.5	3/4	75

[1] Log_2 of average reciprocal antibody titres at 6 weeks of immunization.

[2] Initial inoculum was 5×10^4 or 4.7 log_{10} CFU. Each number is the spleen count for a single mouse.

Fig. 1: HUMORAL RESPONSE (IgG) OF Balb/c MICE TO BRUCELLA ABORTUS ANTIGEN OPS

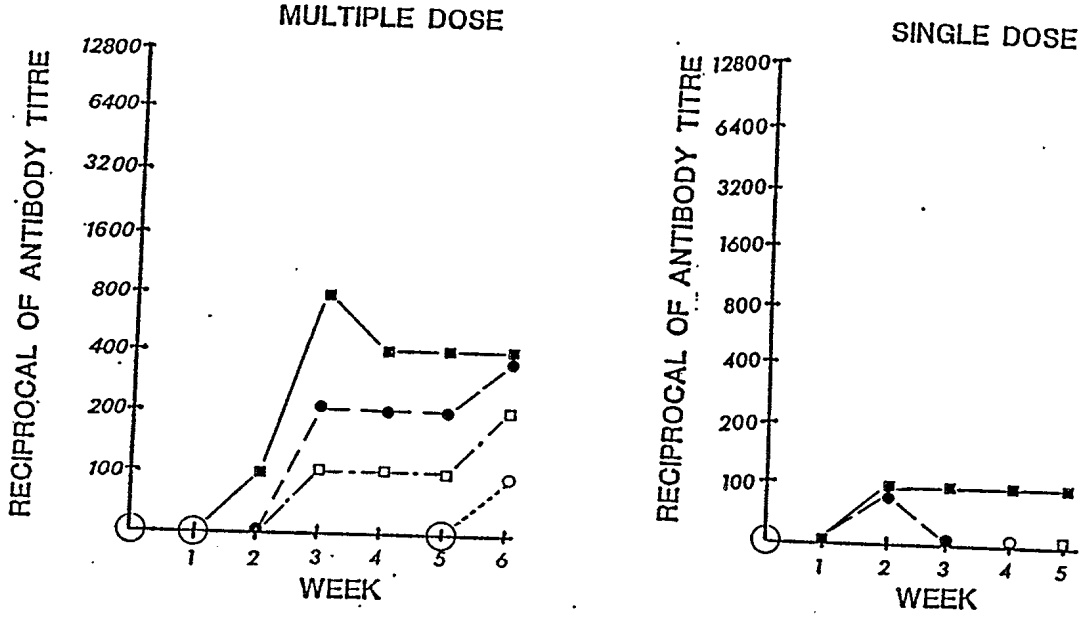

Fig. 2: HUMORAL RESPONSE (IgM) OF Balb/c MICE TO BRUCELLA ABORTUS ANTIGEN OPS

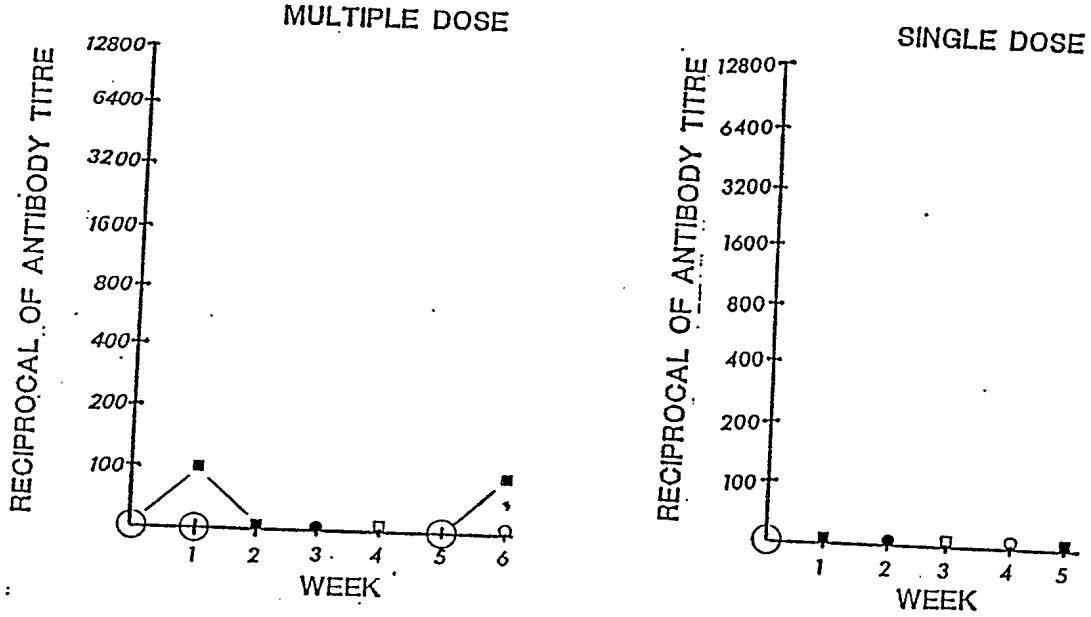

Fig. 3: HUMORAL RESPONSE (IgG) OF Balb/c MICE TO BRUCELLA ABORTUS ANTIGEN LPS

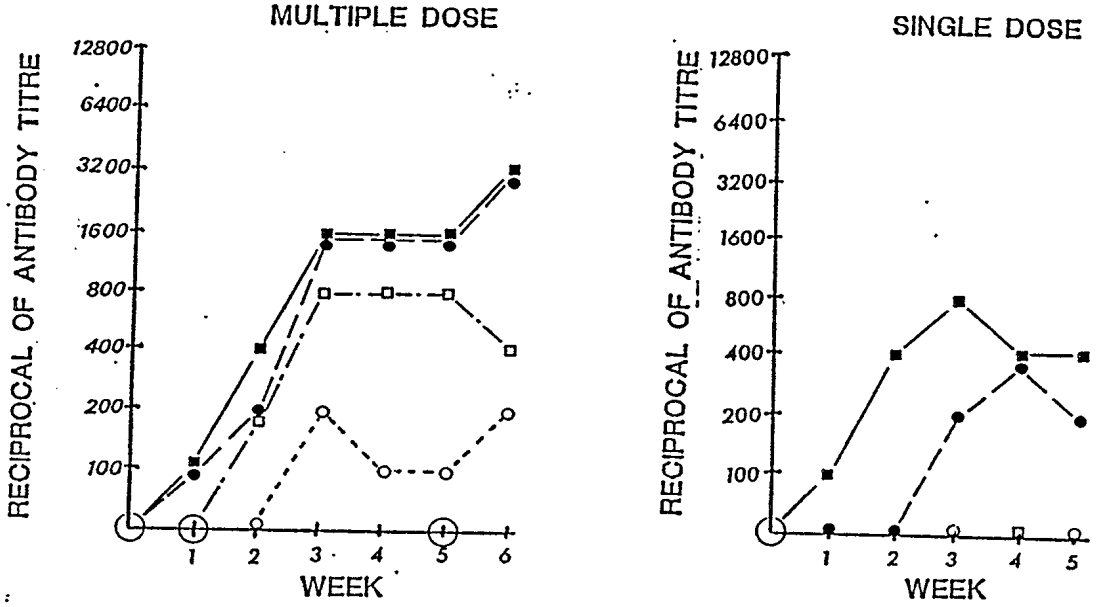

Fig. 4: HUMORAL RESPONSE (IgM) OF Balb/c MICE TO BRUCELLA ABORTUS ANTIGEN LPS

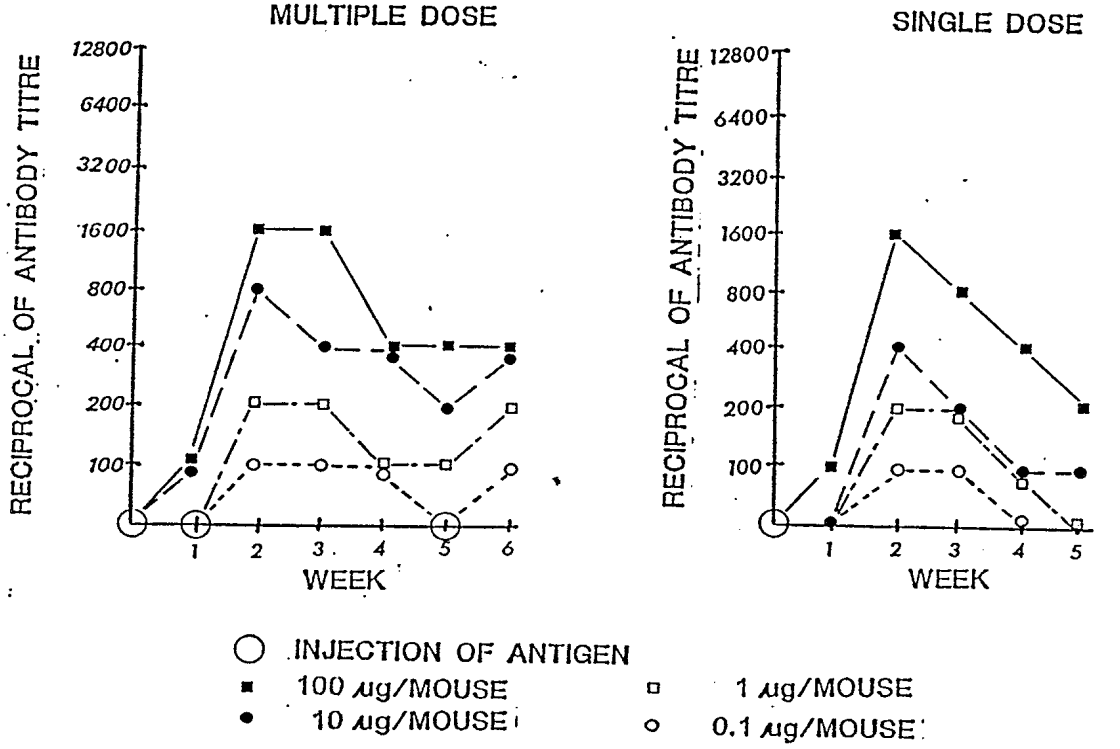

Fig. 5: HUMORAL RESPONSE (IgG) OF Balb/c MICE TO BRUCELLA ABORTUS ANTIGEN OPS-LIPOSOME

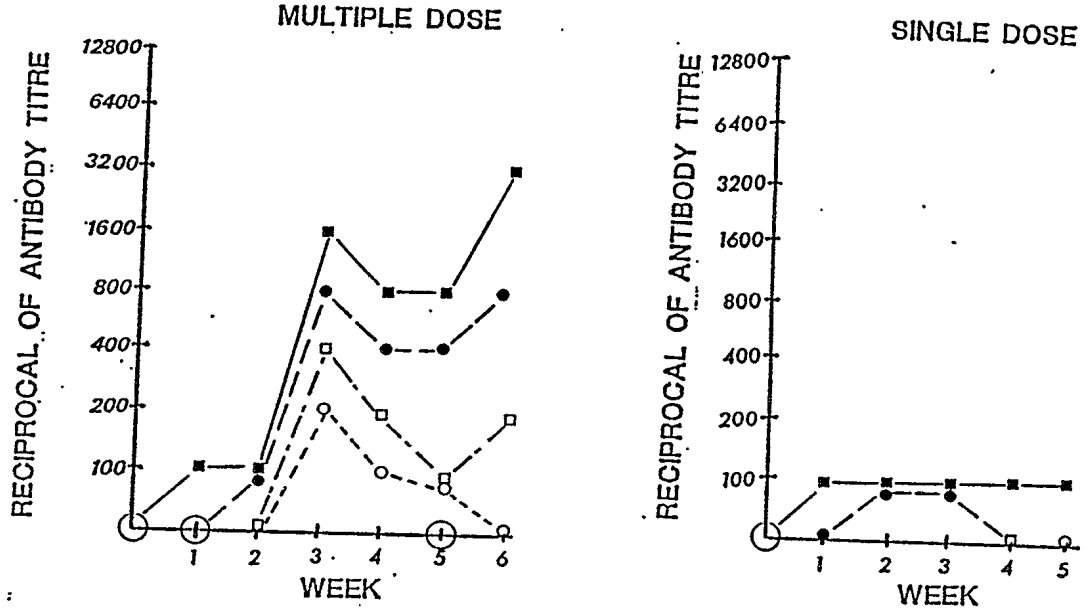

Fig. 6: HUMORAL RESPONSE (IgM) OF Balb/c MICE TO BRUCELLA ABORTUS ANTIGEN OPS-LIPOSOME

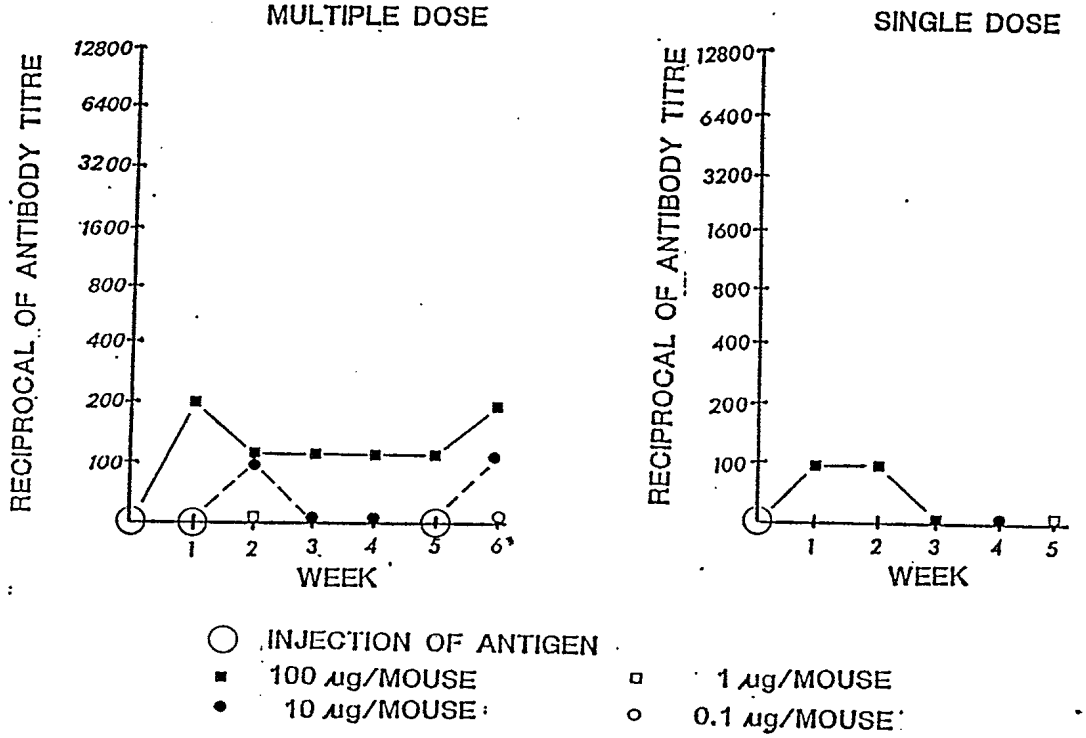

Fig. 7: HUMORAL RESPONSE (IgG) OF Balb/c MICE TO BRUCELLA ABORTUS ANTIGEN LPS-LIPOSOME

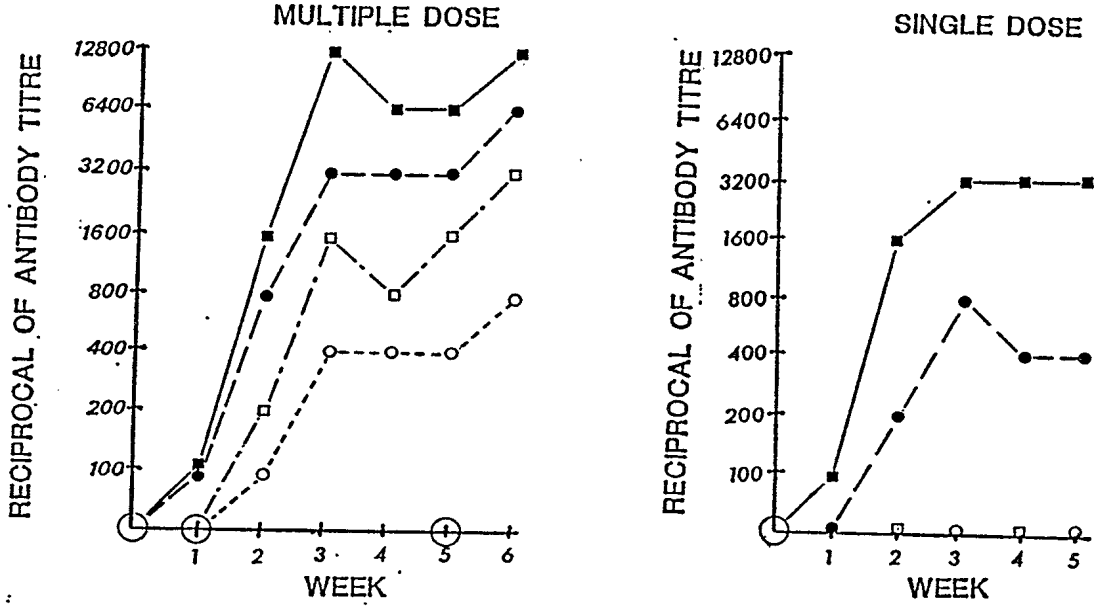

Fig. 8: HUMORAL RESPONSE (IgM) OF Balb/c MICE TO BRUCELLA ABORTUS ANTIGEN LPS-LIPOSOME

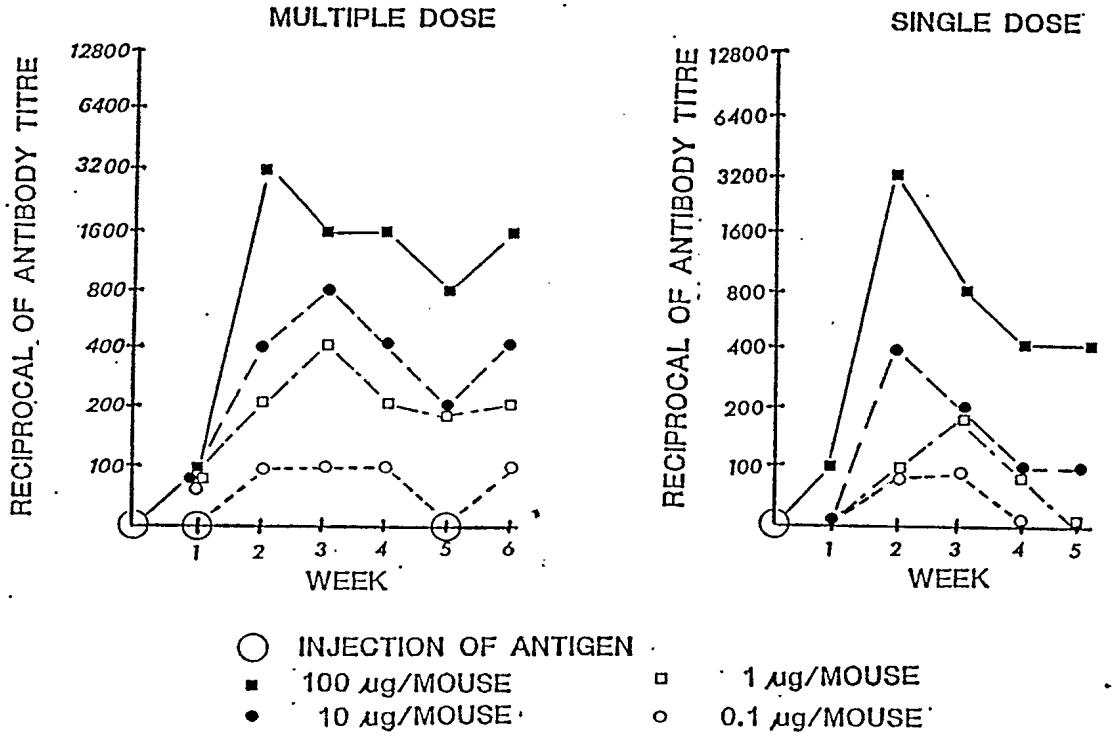

Table II presents data on mice immunized 3 times with the noted concentration of purified antigens then challenged with *B. abortus* 2308. Table III compares mice immunized either once or 3 times with the noted concentrations of purified antigens then challenged with *B. abortus* 30 (another infectious strain that was isolated from an aborted bovine fetus by Agriculture Canada several years ago). Other studies such as those with mice in Chile and with guinea pigs in Colombia, appear to suggest that there is an element of randomness in protection studies, likely due to individual susceptibility or resistance to brucellosis. Although this is apparent as well in our studies presented inTables II and III, general trends can be observed:

- OPS appears to be more effective than LPS as a vaccine
- OPS does not require liposomal encapsulation to be effective, indeed liposomal encapsulation seems to be counter-effective
- a single dose of OPS appears to be more effective than multiple doses of OPS
- the poorer the antibody response to a given antigen (e.g. OPS), the better the protection

Table III: Single vs Multiple Injections of Antigens as Vaccines in the Protection of Balb/c Mice Against *B. abortus* 30.

	Single Injection of Antigen		Multiple Injections of Antigens	
ANTIGEN	Spleen counts (log10 c.f.u.)	Protection (no./total) (%)	Spleen counts (log10 c.f.u.)	Protection (no./total)(%)
Control (none)	4.78,5.20,5.69, 4.59, 4.00	0/5 (0%)	see previous column	see previous column
LPS				
100 ug	3.48, 0, 3.48	1/3 (33%)	0, 5.20	1/2 (50%)
10 ug	3.84, 4.30, 0	1/3 (33%)	4.81,3.60,3.30	0/3 (0%)
1 ug	0, 0, 8.0	2/3 (66%)	0, 4.61	1/2 (50%)
0.1 ug	4.15,5.08,5.78		0, 5.34	1/2 (50%)
Liposomal LPS				
100 ug	0,0	2/2 (100%)	0, 0, 4.82	2/3 (66%)
10 ug	0, 3.60, 0	2/3 (66%)	0, 0, 0	3/3 (100%)
1 ug	0, 5.11	1/2 (50%)	0, 5.36	1/2 (50%)
0.1 ug	0,5.25,3.0	1/3 (33%)	0, 0	2/2 (100%)
OPS				
100 ug	0,0,0	3/3 (100%)	0, 4.40	1/2 (50%)
10 ug	0,0	2/2 (100%)	3.60,3.30,0	1/3 (33%)
1 ug	0,0	2/2 (100%)	0,0	2/2 (100%)
0.1 ug	0,0,0	3/3 (100%)	3.00,5.23	0/2 (0%)
Liposomal OPS				
100 ug	8.18, 0	1/2 (50%)	0,0,0	3/3 (100%)
10 ug	3.84,4.20,0	1/3 (33%)	0,0,0	3/3 (100%)
1 ug	3.85,3.48,0	1/3 (33%)	5.62,5.11,4.28	0/3 (0%)
0.1 ug	0,0	2/2 (100%)	0,0,5.04	2/3 (33%)

Mice were immunized on week 1 for single injection, weeks 1,2 and 5 (intra-muscular) for multiple injections. On week 7 mice were challenged with 5 X 10^4 (log10 of 4.70) of *B. abortus* 30, on week 8 the mice were sacrificed and their spleens assayed for bacteria.

2) Guinea Pig Studies (Colombia):

Dr. Olga Marino of the Instituto Colombiano Agropecuario (ICA), Bogota, Colombia, did an independent investigation on the protective properties of the OPS vaccine. Guinea pigs were used as these are perhaps the most sensitive animal species to *Brucella* infections (Garcia-Carillo, 1990). A vaccine that is protective to these susceptible animals is likely to be protective for humans.

Results for the first set of experiments are not available, although it was reported that 1 mg of OPS was able to protect a 400 g guinea pig from a challenge of 5 X 104 cells of *B. abortus* 2308. At the United Nations University Brucellosis Researchers Network meeting in Valdivia, Chile (April, 1995), another study was presented as noted in Table IV. Results were said to be similar to that of before, except that previously 1000 ug was 100% protective while 3 injections of 1000 ug was only partially effective.

The similarities between the two studies suggest that OPS is protective for guinea pigs against *Brucella* infection, that single doses are more protective than multiple doses, and that protection appears to be inversely related to antibody production (reported but not proesented).

Table IV: Guinea Pigs Immunized with OPS and Challenged with B. *abortus* 2308

Antigen	Infected Animals/Total	% Protection
None (controls)	6/6	0%
Single Dose of OPS		
10 ug	1/4	75%
100 ug	1/4	75%
1000 ug	3/4	25%
Three Doses of OPS		
3 X 10 ug	3/4	25%
3 X 100 ug	3/4	25%
3 X 1000 ug	0/4	100%

3) Swine Study in Venezuela:

In Venezuela, swine are infected not with *Brucella abortus* but with *Brucella suis*, a more infectious species of *Brucella* than the former. The disease is sexually transmitted, passed from an infected boar to a susceptible sow at breeding.

In the presented studies, sows were either left as controls or were vaccinated with different doses of potential vaccines. The swine were cared for 6 months, then both the vaccinates and the controls were mated with the same 4 infected boars to ensure ensemenation and infection. The animals were housed in the same general area on a farm and could be identified by ear tags.

Table V gives a brief summary of the results. It was found that:

- A single dose of 100 ug of OPS (from *B. abortus*) was 100% effective in protecting the sows from *B. suis* infection. Protected swine did not have significant serum titres to *Brucella*. Not only did the pregnancies come to a successful term, but the litter size averaged 11-12 robust piglets.
- For the controls, 68% sero-converted with high titres to *Brucella*, and of these 45% aborted. For control sows that did come to term, 5% had still-born piglets in their litters. For the remainder, although the litter appeared healthy, the average size was 5-6 piglets.

Table V: Venezuelan Swine Study for Vaccinated and Control Sows Challenged with *Brucella suis*

Number	Vaccine	Amount	Dose	Result	1 year later
10	Bab-OPS	100ug	1	no abort.,sero-	protected
10	Bab-OPS	500ug	1	no abort.,sero+/-	protected
10	Bab-OPS	100ug	3	no abort.,sero-	protected
10	Bab-OPS	500ug	3	no abort.,sero+/-	protected
10	Bsu-OPS	100ug	1	no abort.,sero-	protected
10	Bsu-OPS	500ug	1	no abort.,sero+/-	protected
10	Bsu-OPS	100ug	3	no abort.,sero+/-	protected
10	Bsu-OPS	500ug	3	no abort.,sero+/-	protected
10	Bsu-cell	100ug	1	no abort.,sero+	protected
10	Bsu-cell	500ug	1	no abort.,sero+	protected
10	Bsu-cell	100ug	3	no abort.,sero+	protected
10	Bsu-cell	500ug	3	no abort.,sero++	protected
10	RB51	10^6	1	no abort., sero-	protected
10	RB51	10^7	1	no abort., sero-	protected
10	RB51	10^8	1	no abort.,sero-	protected
10	RB51	10^9	1	no abort.,sero-	protected
30	Controls	68% sero+,31% abort			25%abortions

Bab-OPS is *Brucella abortus* 1119-3 O-polysaccharide vaccine, Bsu-OPS is an O-polysaccharide vaccine produced in Venezuela from *B. suis*, Bsu-cell is *B. suis* cells killed with 2% phenol, RB51 is an attenuated live vaccine strain of *B. abortus* from Dr. G. Schurig, Blacksburg, West Virginia.

4) Production of 150,000 "Human Equivalent Doses" of Vaccine

If 100 ug of the OPS vaccine can protect a 25 kg sow from a highly virulent strain of *B. suis*, it is likely that 300 ug of the same vaccine will protect a 75 kg person. Initially we were planning on producing enough *B. abortus* cells and from this enough OPS vaccine to supply the amounts required for collaborative studies with our allies. However, as DRES has had its Level 3 Containment suites under renovations during the term of this task, killed *B. abortus* cells were acquired from external sources. The 2 sources were:

a) Dr. Eduardo Aycardi, Research Manager,
Mr. Oscar Robin, Production Manager,
VECOL,
Empresa Colombiana de Productos Veterinarios,
D.C. Calle 26 (Av. El Dorado), No. 82-93,
Bogota, Colombia.
Tel. 57-1-263-3100
Fax: 57-1-263-8331
(for 2 shipments, 1 kg. + 3.5 kg of *B. abortus* 1119-3 cells killed with 2% phenol, for $15000 US)

b) Drs. Joan Arnoldi, Linda Slater and Janet Payeur,
United States Department of Agriculture,
National Veterinary Services Laboratory,
1800 Bayton Road,
Ames, Iowa,United States, 50010
Tel. 515-239-8568
Fax 515-239-8397
(for 1 kg. of *B. abortus* 1119-3 cells killed with 2% phenol for $1500 US)

Table VI gives a summary of my experience in obtaining cells from these 2 sources. Two other possible sources of killed *Brucella* cells that I have come across since these purchases are the Universidad Austral de Chile (Valdivia, Chile) and ProNaBiVe (Production of National Biologicals for Veterinarian reagents) in Mexico City, Mexico.

Table VI: Comparison of Killed *Brucella abortus* 1119-3 Cells from Different Sources

CRITERIA	VECOL (Bogota, Colombia)	USDA (Ames, Iowa, USA)
Promptness of delivery	**Poor**, it took about 18 months for delivery	**Excellent**, shipment was delivered within 3 weeks of request
Clearance through customs	**Poor**, it was held up at customs in Miami for several weeks due to source being from Colombia	**Excellent**, there were no problems in shipment from the US to Canada
Cost per kg killed cells	**Good** , $15,000 US for 4.5 kg or $3,333/kg	**Excellent**, $1500 US for 1 kg wet weight of cells
Amount of cells delivered	**Poor**, after centrifugation there was about 0.75 kg for each 1 kg claimed to be sent	**Excellent**, after centrifugation there was 0.95 kg cells for the 1 kg claimed
Quality of cells	**Poor**, the first kg sent was clumpy and yellow (probably autoclaved)	**Excellent**, cells were typical tan coloured, creamy in appearance
Yield of OPS	**Good**, about 8 g OPS/800 g wet weight of cells (1% yield)	**Excellent**, about 30 grams OPS/0.95kg cells (about 3.2 % yield)

The OPS vaccine was then extracted from the above cells using the "Rapid Method" reported by Cherwonogrodzky et al., 1990. The method is that noted in Table VII. It should be noted that Lot#1 differs from Lot #2 in that the former used autoclaving as a source of heat for the hydrolytic release of polysaccharide while the latter used a boiling water bath. For Lot#1, the cells were first washed and resuspended in 1% NaCl, 2% acetic acid. The cells were then autoclaved but due to a malfunction the conditions were 140°C instead of 121°C, the pressure was about 23 psi instead of 15 psi, and the time was about 1 hr instead of 30 min. Charing and yellowing of the OPS was observed, although the Colombian study with guinea pigs suggests that this did not seriously affect the potency of the vaccine. For the Lot#2 extractions, the cells were washed and resuspended as before in 1% NaCl, 2% acetic acid, but instead were heated in a boiling water bath (99°C) for 2 hours. The yield of OPS was less (8 grams instead of 30 grams per kg), but there was less charing and less yellowing of the vaccine. A total of 45 grams (150,000 human equivalent doses) has been purified for research and experimental purposes.

Table VII: Procedure for OPS Vaccine Extraction

Part A: Cells are centrifuged, the supernatant is collected, the cells are resuspended in 1% NaCl, recentrifuged, and the supernatants are pooled. This liquid has acetic acid added to a final v/v of 2%. The liquid is heated (i.e. the flask with the liquid is placed in a boiling waterbath for 2 hrs, a magnetic stirring bar in the liquid is used for continuous mixing) then cooled. 2 M tricholoroacetic acid (TCA) is added to a final concentration of 0.2 M TCA and chilled 4°C overnight. The preparation is centrifuged and the precipitate discarded. The supernatant is extracted with liquid phenol, the mixture is chilled, and the phenol removed. The OPS is precipitated and the phenol is removed with 3 washes of methanol with 1% sodium acetate. The OPS is redissolved in water, then dialyzed (Spectapor 1000 m.w. dialysis tubing) against 2 changes of distilled water and one of 0.4% pyridine/0.4% acetic acid (pH 4). There is another centrifugation to remove debris and the supernatant with the OPS vaccine is lyophilized.

Part B: The cells from Part A are resuspended in at least 5 volumes of 1% NaCl / 2% acetic acid. The suspension is heated as before, centrifuged and the supernatant is treated as before.

Part C: The cells from Part B are resuspended as before, heated then centrifuged and the supernatant is treated as before.

Future requirement for this vaccine are:

- to redissolve the entire amount in 0.4% pyridine, 0.4% acetic acid (pH 4) buffer and elute this through a G50 Sephadex column (the dextran beads will adsorb out any trace amounts of LPS or pyrogen and will remove any particulate material)
- filter sterilize the eluted vaccine (ie. with a 0.45 um Millipore filter unit)
- aseptically dispense it into sterile labelled vials (10 doses or 3 mg OPS/vial)
- the vaccine should be freeze-dried and the vials aseptically sealed
- the vaccine should be stored in a cool dark place (under these conditions it should be stable for at least 10 years.

Preliminary Evidence that the OPS Vaccine is Better Than Anticipated:

1. One of the most encouraging results is the absolute protection that the OPS vaccine gave swine against *B. suis* infection. Not only did it protect, but given that the polysaccharide was from *B. abortus* but protected swine from *B. suis*, it is likely that there is broad protection against *Brucella* species.
2. The hope was that the vaccine would protect swine from brucellosis. Not only were small amounts (i.e. 100 ug) of vaccine protective, but a single injection protected swine that were exposed to disease 6 months later. Also, a year after these studies were done, these same swine were protected from any incidence of infection (i.e. the vaccine is long lasting). Curiously, the farm where these swine were kept had an epidemic of *Haemophilus pleuropneumonia*. Unlike the rest of the swine, those immunized with the OPS vaccine remained healthy. It is unknown if cross-protection has occured or whether the vaccinates were spared by chance.
3. Dr. Kournikakis at DRES has found that the OPS vaccine may be a powerful immuno -modulator, enhancing general immunity against disease. This work is ongoing.
4. Routinely, mice are challenged with about 10^4 virulent *Brucella* cells, which is about 1000 LD50s. In an attempt to overwhelm immunity, Dr. Rojas in Chile infected mice with 10^9 virulent *B. abortus* 2308 cells (100,000,000 LD50s) and found partial protection (some mice did not get infected though others did) for the animals given the OPS vaccine.
5. Due to the lack of Level 3 Containment facilities, studies could not be done to determine

if the OPS vaccine can protect animals from bacteria that cross-react with *Brucella*. However, the OPS structure of *Francisella tularensis* was recently found to be similar to that of *Brucella* (Cherwonogrodzky et al., publication in preparation) and hence gives an encouraging support for this concept.

6. The identical OPS of *B. abortus* is found on *Yersinia enterocolitica* O:9, and the identical OPS of *B. melitensis* is found on *Escherichia hermannii*. It is likely that these latter less virulent strains may serve as a ready source of OPS vaccine.
7. The up-coming "competition" with the OPS vaccine is an attenuated rough mutant of *B. abortus* 2308 called RB51, developed by Dr. G. Schurig of Blacksburg, West Virginia. The mechanism of its protection is unknown, but I suspect that this strain is rough because it can synthesize and secrete the OPS (hence providing a source of the same DRES vaccine *in vivo*), but it is defective in linking the polysaccharide to its LPS.

Immediate Action Required:

The OPS vaccine is novel and has been shown to be effective in the protection against brucellosis. It should be patented for DND's ownership.

Cost Analysis of Task 0518B-11, DMO-007

Amount provided by the Surgeon General's Office.....	160,000
Amount spent..... (deficit absorbed by Medical Counter-measure Section)	193,000
Deliverables*:	
150,000 human equivalent doses of vaccine.....	150,000
Mouse studies (DRES).....	26,000
Mouse studies (Chile).....	5,000
Guinea pig studies (Colombia).....	16,000
Swine trials (Venezuela).....	225,000
OPS from *Y.enterocolitica* O:9 from NRC (Ottawa).....	5,000
OPS from *Escherichia hermannii* from NRC (Ottawa).....	5,000
Equipment (centrifuge, etc.).....	48,700
TOTAL	$480,700

* The deliverables are estimates of what it would take to prepare reagents or conduct studies within Canada. Many of the studies were perfomed in South America at a fraction of these costs, the reagents from NRC were provided as a gift.

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CHERWONOGRODZKY, JOHN W.

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TASK 0518-11: DMO-007

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From 30 August 1991 to 30 August 1995, DRES received a task from the Surgeon General's Office to develop a liposomal vaccine both to *Brucella abortus* and *Francisella tularensis*. Funding was $160,000 over these 4 years. Currently there is no vaccine for human use to the *Brucella* species. There is an effective attenuated vaccine to tularemia, *Francisella tularensis* live vaccine strain (LVS), but this bacterium can revert to a virulent form if cultural conditions are not followed as specified.

A novel purified polysaccaride vaccine has been produced for *B. abortus*. Liposomal encapsulation was found not to be required for its effectiveness. Protection did not appear to be due to an antibody response, and indeed may be counter-indicative. The vaccine was protective for mice and guinea pigs against *B. abortus* and for swine against *B. suis* (hence it appears to offer broad protection). Single injections of 100 ug (lesser amounts were not tested) were found to be 100% protective for 25 kg swine challenged with virulent field strains of *B. suis*. A year later these swine were still protected against brucellosis. Based on these results it was extrapolated that 300 ug might protect a 75 kg person. Initially 15,000 human dose equivalents was to be prepared for research and a potential emergency supply. The final preparation was enough for 150,000 human equivalent doses. Due to a lack of containment facilities, the vaccine could not be tested for protection against cross-reactive pathogens (e.g. *Francisella tularensis*).

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