Papers and Articles

Organochlorine and mercury contamination in United Kingdom seals

M. P. Simmonds, P. A. Johnston, M. C. French

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In 1988 and 1989 tissue samples were obtained from the grey seal (*Halichoerus grypus*) population found in the Dee estuary in the north west of England and from harbour seals (*Phoca vitulina*) from the populations in the Wash and north east Scotland and analysed for mercury and organochlorine compounds. Adult seals from the Dee estuary were highly contaminated with mercury and polychlorinated biphenyls (PCBs), and one animal from the Dee contained traces of dichlorodiphenyltrichloroethane (DDT), suggesting the recent use of this banned pesticide. The levels of hexachlorobenzene in the livers of two Dee seals exceeded those in the blubber, possibly indicating liver malfunction or recent exposure. The same relationship was found for hexachlorobenzene in three specimens from the Wash and, in one of these animals, the liver was also more highly contaminated than the blubber with dieldrin and PCBs. Levels of contamination were lower in seals from the Wash and even lower in animals sampled in Scotland, where only dichlorophenyldichloroethylene, the metabolite of DDT, was routinely detected. The toxicological significance of the results is discussed, particularly in relation to the mortality observed in the seal epizootic of 1988.

THE significance of environmental pollution for marine mammals has long been an issue of concern. Metals, pesticides and related xenobiotic chemicals, in particular polychlorinated biphenyls (PCBs), are known to accumulate in these marine predators (Holden and Marsden 1967). Mercury has no known biological function and mercury salts are acutely toxic to man and higher animals (Goyer 1986). Low molecular weight alkyl-mercury compounds have a high chronic toxicity, and result in irreversible changes in nerve tissue. Methyl mercury is important in this regard because it is produced from ionic mercury by the activity of bacteria and can be accumulated by fish (Steinnes 1990).

Reproductive failure in Baltic and Wadden Sea seals have been correlated with the presence of PCBs and dichlorodiphenyltrichloroethane (DDT) (Reijnders 1980). It has been shown experimentally that the consumption of PCB-contaminated fish reduced the reproductive success of harbour seals (Reijnders 1986) and reduced the blood levels of retinol (a precursor of vitamin A) and thyroid hormones (Brouwer and others 1989). Moreover, laboratory studies have demonstrated a wide range of possible effects of exposure to organochlorine compounds, including immunosuppression, reproductive impairment and liver damage (Safe 1984). It has been predicted that fish-eating marine mammals could become extinct unless the input of organochlorine chemicals into the oceans is halted (Klamer and others 1991).

A morbillivirus, phocine distemper virus (Mahy and others 1988) was the proximate cause of heavy seal mortality in northern Europe in 1988, and the opportunity was taken to collect tissue samples in order to examine regional variations in the level of contamination.

The largest congregations of European harbour seals, *Phoca vitulina*, occur around the United Kingdom and almost all of Europe's grey seals, *Halichoerus grypus*, reproduce in British waters (Reijnders 1989, Harwood 1990). On the basis of the estimated inputs of contaminants into these waters (Dickson 1987, Anon 1987) different populations would be expected to be exposed to different levels of contamination of their environment. This paper presents data on the burdens of contamination in seals from three British sites and considers their significance.

Materials and methods

Blubber samples from the mid-ventral region and liver and kidney samples were obtained from wild harbour seals which died during the epizootic of phocine distemper virus. The animals originated from the colonies which haul out on the sand banks of the Wash or nearby, off the north Norfolk coast. Before they died they were held in the Seal Assessment Unit at Docking, Norfolk. A similar range of samples was obtained from grey seals from the population found in the Dee estuary, on the north west coast of

TABLE 1: Concentrations of mercury (ppm dry weight) and DDE (ppm wet weight) in blubber samples from seals which died during the seal epizootic of 1988-89 on the east coast of Scotland

Seal	Sex	Age	Length (cm)	Location	Date sampled	Hg	DDE
A1	M	SA	127	Cruden Bay	25/8/88	ND	0·99
A2	F	SA	119	Peterhead	29/10/88	0·123	0·70
A3	M	Y/P	106	Aberdeen	11/9/88	ND	ND
A4	M	Y/P	91	Peterhead	13/9/88	ND	3·96
A5	M	Y/P	107	Boddam	28/9/88	ND	1·68
A6	F	NK	119	Aberdeen	21/10/88	ND	ND
A7	F	Y/P	99	Peterhead	29/10/88	0·146	2·62
A8	F	Y/P	112	Balmedie	17/10/88	ND	1·53
A9	M	Y/P	117	Boddam	19/10/88	ND	0·82
Mean values						0·03	1·37

NK Not known, ND Not detected, SA Sub adult, Y/P Yearling or 1988 pup
HCB, dieldrin, TDE, DDT and HCH were not detected
PCBs were detected only in seal A7 blubber, at 1·03 ppm
All the carcases were fresh when sampled, and all were infected with PDV (L. T. A. Brain, personal communication)
Seals A4 and A7 had thin blubber layers (L. T. A. Brain, personal communication)

M. P. Simmonds, BSc, CBiol, MIBiol, School of Environmental Sciences, The University of Greenwich, Creek Road, Deptford, London SE8 3BU
P. A. Johnston, BSc, PhD, Earth Resources Centre, University of Exeter, Laver Building, North Park Road, Exeter EX4 4QE
M. C. French, PhD, CBiol, MIBiol, MRSH, Institute of Terrestrial Ecology, Monks Wood Research Station, Abbots Ripton, Huntingdonshire PE17 2LS

TABLE 2: (a) Concentrations of mercury (ppm dry weight) and organochlorine compounds (ppm wet weight) in liver (L), kidney (K) and blubber (B) samples from Norfolk harbour seals which died during the seal epizootic of 1988-89

Seal	Hg			HCB			DDE			Dieldrin			PCBs			HCH		
	L	K	B	L	K	B	L	K	B	L	K	B	L	K	B	L	K	B
B1	136·3	11·8	0·296	0·13	0·03	0·09	0·16	0·18	1·51	ND	ND	ND	4·14	2·32	21·1	ND	ND	ND
B2	21·2	7·98	0·269	ND	ND	ND	ND	ND	1·97	ND	ND	ND	ND	ND	ND	ND	ND	ND
B3	13·5	2·92	0·129	ND	ND	ND	ND	ND	2·19	ND	ND	ND	ND	ND	ND	ND	ND	ND
B4	2·71	1·92	ND	ND	ND	ND	ND	ND	1·11	ND	ND	ND	ND	0·75	ND	ND	0·23	0·35
B5	1·62	1·50	0·058	ND	ND	0·01	ND	ND	0·31	ND	ND	ND	3·54	0·76	7·32	ND	ND	ND
B6	4·91	6·16	0·123	0·18	0·03	0·07	0·22	0·05	1·35	0·33	ND	0·13	11·1	2·04	8·89	ND	ND	ND
B7	3·93	2·05	0·056	0·09	ND	0·02	0·24	0·10	1·78	0·13	0·12	0·30	8·25	3·26	45·8	ND	ND	ND
B8	2·04	2·38	ND	ND	ND	ND	ND	ND	0·94	ND	ND	ND	ND	ND	ND	ND	ND	ND
B9	2·95	1·92	0·131	ND	ND	ND	ND	ND	1·12	ND	ND	ND	ND	0·55	0·54	ND	0·12	0·13
B10	3·17	3·40	0·129	ND	ND	ND	ND	ND	2·45	ND	ND	ND	ND	ND	ND	0·27	0·28	0·28
B11	15·2	9·30	0·242	ND	ND	ND	ND	ND	9·67	ND	ND	ND	3·05	ND	16·65	ND	ND	0·29
B12	5·94	5·85	0·166	ND	ND	ND	ND	ND	3·47	ND	ND	ND	ND	1·03	ND	ND	ND	ND
B13	7·30	5·04	0·104	ND	ND	ND	ND	ND	1·09	ND	ND	ND	ND	ND	ND	ND	0·25	0·23
B14	7·44	4·95	1·26	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0·56	0·55
Mean	16·3	4·8	0·212	0·029	0·004	0·014	0·044	0·023	2·07	0·033	0·009	0·031	2·15	0·77	7·17	0·019	0·010	0·131
B15	5·62	NS	0·336	ND	NS	ND	ND	NS	0·6	ND	NS	ND	ND	NS	ND	ND	NS	ND
Law and others (1989)						0·004			3·1						23			

ND Not detected, NS Not sampled
Seals B1 to B14 were from the Seal Assessment Unit, Docking, Norfolk, and seal B15 was from the Kings Lynn Seal Sanctuary; their bodies were fresh when sampled
TDE was detected only in seal B1 (blubber 0·29 ppm)
DDT was detected only in seal B1 (liver 1·12 ppm, blubber 3·29 ppm) and seal B7 (blubber 0·85 ppm)

England, which were found dead on the shore. Blubber samples were also obtained from harbour seals which died on the east coast of Scotland, between Aberdeen and Fraserburgh. The harbour seal samples were taken between September and December 1988 and the grey seal samples were collected in August 1988 and between May and July 1989. The sampling dates are given in Tables 1, 2 and 3. All the samples were wrapped in pesticide residue analysis-grade-hexane-washed aluminium foil, placed in similarly washed glass vials and transferred frozen to the laboratory where they were stored at –20°C before analysis.

The sex and approximate age of the seals were ascertained by visual inspection. It was not possible to differentiate between pups born in 1988 and yearling animals. Where they are known, the mid-ventral blubber thickness, the length from nose to tip of tail and the condition of carcases sampled are reported. All the seals from Norfolk and Scotland were recently dead when sampled; those from the Dee were in variable condition (Tables 1, 2 and 3).

TABLE 2: (b) Details of the harbour seals sampled at the Seal Assessment Unit, Norfolk

Seal	Sex	Age	Length (cm)	Date sampled	Length of captivity	Blubber depth (cm)
B1	F	Adult	121	3/10/88	Few hours	1·4
B2	F	Y/P	102	6/10/88	Few hours	1·3
B3	F	Y/P	117	30/10/88	15 days	3·5
B4	M	Y/P	120	13/11/88	8 days	2·0
B5	M	Y/P	95	18/10/88	19 days	3·0
B6	M	Y/P	93	20/10/88	12 days	1·0
B7	F	Y/P	97	29/10/88	13 days	1·5
B8	F	Y/P	102	1/11/88	33 days	1·7
B9	M	Y/P	100	2/11/88	18 days	3·5
B10	M	Y/P	96	31/10/88	16 days	1·0
B11	F	Y/P	89	13/11/88	20 days	1·5
B12	M	Y/P	95·5	5/11/88	13 days	1·5
B13	M	Y/P	101	26/11/88	14 days	2·0
B14	M	Y/P	97	9/12/88	1 day	1·7
B15	F	Y	98	7/8/88	1 year	-

Y/P Yearling or 1988 pup, Y Yearling

Mercury analysis

Approximately 2 g of tissue were weighed into a boiling tube and dried for 72 hours at 85°C. The dried sample was digested for 12 hours in 10 ml of cold analytical grade nitric acid and then boiled for one hour. The digests were made up to 25 ml and analysed by cold vapour generation, according to the method of Hatch and Ott (1968), on a Thermo-electron 151 atomic absorption spectro-photometer. Normal quality control procedures were used to check the accuracy and precision of the results obtained. The detection limit was 4 ppb wet weight.

Organochlorine analysis

Approximately 0·7g of tissue was homogenised, using a mixture of acid washed sand and anhydrous sodium sulphate as a grinding agent. Both reagents had previously been baked at 700°C for five hours. The homogenates were allowed to soak overnight in a 1:1 mixture of pesticide analysis residue-grade acetone and hexane, and extracted repeatedly with the solvent mixture into a final volume of 50 ml. The extracts were shaken and allowed to stand overnight, after which 25 ml were pipetted into a pre-weighed universal bottle and evaporated to constant dry weight to give the lipid content of the sample. The lipid sample was then redissolved in 5 ml of hexane and shaken occasionally for one hour.

The sample was cleaned by passing 1 ml of the extract through a packed column containing aluminium oxide which had previously been held at 800°C for four hours and de-activated by tumbling for one hour after the addition of 5 per cent distilled water. The column was eluted with 1 ml aliquots of hexane until 5 ml of cleaned extract was obtained. The sample was analysed on a Varian 3400 gas chromatograph fitted with a 30 m DB210 capillary column, temperature programmed to 190°C. The components were identified and measured by comparison with a standard pesticide mixture and a PCB standard of Aroclor 1254. The following pesticides were analysed in the samples from all three sites: hexachlorobenzene, dieldrin, γ-hexachlorocyclohexane (lindane) and dichlorophenyldichloroethylene (DDE) (the metabolite of DDT). Tetrachlorodiphenylethane (TDE) and DDT were determined in the samples from the Dee seals. PCBs were determined in all the samples. Recoveries were tested on spiked samples and always exceeded 95 per cent. The detection limits for the organochlorine compounds were: hexachlorobenzene (HCB) and γ hexachlorocyclohexane (HCH) 0·005 ppm; DDE, DDT, TDE and dieldrin 0·01 ppm; and PCBs 0·05 ppm wet weight.

Results

Details of the seals sampled and the results of the analyses are given in Tables 1, 2, 3 and 4. The duration of captivity of the Norfolk seals is also recorded. All the Norfolk and Scottish seals sampled were reported to be infected with phocine distemper virus (D. and C. Clarke, personal communication and L. T. A. Brain, personal communication). The Dee seals died from a variety of causes and, where known, these are given in Table 3. Table

TABLE 3: Concentrations of organochlorine compounds (ppm wet weight) and mercury (ppm dry weight) in samples of liver (L), kidney (K) and blubber (B) from grey seals living in the region of the Dee Estuary

Seal	Sex	Location	Date sampled	Hg			HCB			DDE			Dieldrin			TDE			DDT			PCBs		
				L	K	B	L	K	B	L	K	B	L	K	B	L	K	B	L	K	B	L	K	B
C1	F	Hoylake	6/5/89	4088	62·2	1·18	ND	ND	0·17	0·14*	0·02	0·05	ND	ND	0·18	ND	ND	ND	ND	ND	ND	1·14	0·09	10·17
C2	M	Mostyn	27/6/89	2470	2·02	5·89	0·11*	0·08	0·04	0·09	0·06	ND	0·18	ND	0·64	0·18	ND	1·34	ND	ND	ND	13·61	13·43	80·57
C3	M	West Kirby	21/6/89	819	96·1	0·174	0·03*	ND	0·02	0·21	0·09	0·29	ND	ND	0·56	ND	ND	0·21	ND	ND	1·07	4·15	1·04	116·68
C4	M	Hilbre Island	23/5/89	1310	106·0	0·549	ND	ND	ND	0·28	0·28	3·59	ND	ND	0·30	ND	0·89	0·12	ND	ND	1·13	5·17	2·22	29·64
C5	M	River Dee	15/7/89	-	66·6	0·191	-	0·04	0·32	-	0·01	0·07	-	0·03	0·03	-	ND	ND	-	0·25	1·08	-	2·22	28·96
C6	F	West Kirby	10/7/89	3730	-	3·13	ND	-	ND	0·23	-	4·16	ND	-	0·63	ND	-	ND	ND	-	ND	1·18	-	46·83
C7	F	Thurtaston	3/8/88	51·6	64·9	1·40	0·01	ND	ND	0·27	0·03	0·27	ND	0·07	ND	ND	ND	ND	ND	ND	ND	2·23	0·95	14·65
Mean values				2078	66·30	1·79	0·025	0·02	0·079	0·203	0·082	1·20	0·03	0·02	0·334	0·03	0·148	0·239	0	0·042	0·469	4·58	3·33	46·79
C8	F	Southport	21/8/88	6·81	-	0·498	ND	-	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-	ND

Key as Table 1
All seals were adult except for C7 which was only partly grown and C8 which was a fetus one month from termination (Baker, personal communication)
HCH was detected only in seal C2 Liver at 0·04 ppm, seal C3 Blubber at 0·09 ppm and seal C5 Blubber at 0·01 ppm
Bodies C3 and C4 were fresh, C7 and C8 were moderately decomposed and the rest were in an advanced stage of decomposition
The causes of death of all except C2 and C5 are known (Baker, personal communication). C1 died of asphyxia, resulting from an intestinal blockage, C3 and C4 died of pneumonia, C6 of a trauma to the neck, C7 of a mesenteric granuloma (the animal was also distinctly emaciated) and the aborted fetus, C8, was caused by bacterial infection

4 reports the lipid content of the samples. Unless otherwise stated, the concentrations are in parts per million (ppm) wet weight for organochlorine compounds and ppm dry weight for mercury.

Scottish harbour seals (Table 1)

DDE was detected in seven of nine seals at a mean level of 1·37 ppm. PCBs were found in one blubber sample at 1·03 ppm. Mercury was found in the blubber of seals A2 and A7 at 0·12 and 0·14 ppm, and the blubber layer was thin in seals A4 and A7.

Wash (Norfolk) harbour seals (Table 2)

In the blubber samples taken from the 14 seals held in the Seal Assessment Unit, DDE was found in 13 (mean value 2·07 ppm), PCBs in six (mean 7·17 ppm) and HCB in four (mean 0·014 ppm). Dieldrin was found in two seals' blubber (mean 0·031 ppm) and HCH in six (mean 0·131 ppm). TDE and DDT were detected in the blubber (0·29 ppm TDE and 3·29 ppm DDT) and liver (1·12 ppm DDT) of the only adult animal sampled. Mercury was detected in the liver (mean 16·3 ppm) and kidneys of all the animals sampled and in the blubber of 12. Seal B15 which had been held for a year in a local seal sanctuary before succumbing to infection had detectable levels of mercury (5·62 ppm) in its liver and DDE (0·60 ppm) in its blubber. The data of Law and others (1989) for a similar group of Norfolk seals are included in Table 2 for comparison.

Dee grey seals (Table 3)

The blubber of all seven animals sampled was contaminated with PCBs (mean 46·8 ppm). Blubber from six seals also contained DDE (mean 1·20 ppm) and dieldrin (mean 0·334 ppm). Four were contaminated with HCB (mean 0·079 ppm), three with TDE (mean 0·239 ppm) and DDT (mean 0·469 ppm), and two with HCH (at 0·09 and 0·01 ppm). In the fetus sampled, mercury, presumably transferred across the placenta, was detected at 6·81 ppm in the liver and 0·498 ppm in the blubber. Mercury was detected in all the samples analysed and the concentrations in the livers ranged from 51·6 to 4088 ppm (mean 2078 ppm); for comparative purposes these values may be expressed in terms of wet weight as from 17·35 to 1097 ppm, mean 571·3 ppm.

Discussion

The results show that the Scottish harbour seals had fewer contaminants and lower levels of contamination in their tissues than seals from the Wash. In seals from the Wash, the mean blubber concentration of DDE (2·07 ppm) was almost twice that of the Scottish seals (1·37 ppm) and PCBs were also present in higher concentrations (mean 7·17 ppm compared with a value of 1·03 ppm found in only one of the nine seals sampled). The levels of contamination in the adult Dee seals were much higher than in either group from the east coast, with a mean PCB-blubber concentration of 46·8 ppm. Gross pathological blockages of the uterus, known to be associated with DDT and PCB contamination, have recently been reported from Dee seals (Baker 1989).

In general, the mercury levels appeared to be related to the known inputs to the waters of the coastal areas sampled (Anon 1987, Dickson 1987). There was roughly a 10-fold increase in concentration between the young Scottish harbour seals and the Wash seals of a similar age and there was a similar increase to the levels of contamination in the adult grey seals in the Dee.

The mercury concentrations in the livers of the Dee seals are among the highest reported for seals from any northern waters (mean 571·3 ppm wet weight) and two values (1097 and 758·1

TABLE 4: Hexane extractable lipid expressed as a percentage weight of seal tissues sampled from north east Scotland, The Wash and the Dee estuary

Region															
North east Scotland															
	A1	A2	A3	A4	A5	A6	A7	A8	A9						
Blubber	83·4	94·1	87·6	74·1	62·9	79·9	82·8	71·9	71·7						
The Wash															
	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	B14	B15
Liver	2·87	4·11	2·61	1·74	5·02	2·00	2·29	7·89	2·29	3·42	1·80	2·24	2·45	2·87	4·73
Kidney	3·06	2·17	2·56	2·48	3·37	2·34	2·10	2·04	1·90	1·94	1·72	2·00	3·42	2·40	-
Blubber	64·2	86·1	90·4	95·0	78·9	73·5	83·2	91·7	91·9	87·3	82·4	79·7	91·9	4·14	88·3
Dee estuary															
	C1	C2	C3	C4	C5	C6	C7	C8							
Liver	8·01	12·4	4·27	3·55	-	4·79	9·13	2·06							
Kidney	3·30	6·92	2·34	3·15	2·87	-	5·78	-							
Blubber	55·2	30·8	75·3	42·2	72·7	57·9	32·2	9·63							

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Efficacy of an Australian *Babesia bovis* vaccine strain in Malawi

J. A. Lawrence, J. Malika, A. P. Whiteland, P. Kafuwa

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Three calves vaccinated with the Australian Ka strain of *Babesia bovis* were fully protected against experimental infection with an isolate from a farm on which four of 210 vaccinated cattle had died from *B bovis* infection. A degree of cross protection against the isolate was demonstrated in one calf which had been infected previously with *Babesia bigemina*.

BABESIA bovis is an important tick-borne pathogen of cattle in eastern and southern Africa which occurs in association with its vector, *Boophilus microplus*, along the east coast and for a variable distance inland. The parasite is endemic throughout Malawi. Vaccination to control the disease in improved cattle in Malawi was introduced on a limited scale in 1987, in conjunction with vaccination against other tick-borne diseases, notably East Coast fever (*Theileria parva* infection). In the initial stages, frozen blood vaccine containing the attenuated Ka strain of *B bovis* was imported from the Tick Fever Research Centre, Wacol, Queensland, Australia. Since 1991, vaccine produced in Malawi from Australian seed material has been used. Australian vaccine has been shown to provide good protection over a wide area (Callow 1976) including South Africa (de Vos and others 1982).

Doubts about the efficacy of the Australian vaccine strain in Malawi were raised in 1991 after an outbreak of *B bovis* infection in vaccinated cattle on Likasi Farm near Lilongwe, in the Central Region of Malawi. The farm had experienced a severe outbreak of tick-borne disease in 1988, in which *B bovis* infection was an important component, following an interruption in the dipping programme. The outbreak had been controlled by the reintroduction of weekly dipping in chlorfenvinphos (Supona; Shell Chemicals) and by immunisation, and subsequently tick-borne diseases had been infrequent. In June and July 1991, several deaths occurred in vaccinated cattle from *B bovis* infection. The outbreak was investigated and the organism was isolated to test it against the Ka strain vaccine.

The outbreak

Between May 31 and July 8, 1991, eight cattle on the farm were confirmed by laboratory examination as having died from *B bovis* infection. In most cases, the animals were reported to have been found dead or to have died after a short illness. The deaths followed a period of suspension of dipping between May 13 and June 10 because of a failure of the farm water supply. Ticks were not reported on any cattle during the period. The deaths involved three herds of crossbred Friesian cattle between one and four years old. Younger and older Friesian crossbreds, Malawi Zebus and Brahmans in five other herds were not affected. After the resumption of regular dipping, the outbreak stopped, although one further case occurred in September.

TABLE 1: Mortality from *Babesia bovis* and vaccination status among breeding heifers

	Survived	Died	Total
Vaccinated 1989	110	3	113
Vaccinated 1990	96	1	97
No record of vaccination	48	2	50
Total	254	6	260

TABLE 2: Serological status for *Babesia bovis* and vaccination status of breeding heifers

	Seropositive*	Seronegative	Total
Vaccinated 1989	18	2	20
Vaccinated 1990	19	1	20
Not vaccinated	14	5	19
Total	51	8	59

* Antibodies detected by immunofluorescence at a dilution of 1/90

Investigations revealed that the losses occurred in two groups of cattle, yearling steers and heifers in one herd (three deaths out of 90 animals) and breeding heifers in another two herds (six deaths out of 260 animals). Mortality in the yearlings was not unexpected, as very few had been vaccinated. Mortality in the breeding heifers involved both vaccinated and unvaccinated animals. The majority of the heifers had been vaccinated either in 1989 or 1990 with frozen trivalent anaplasma/babesia vaccine (Combavac; Tick Fever Research Centre). Seroconversion to *B bovis* after the use of this vaccine in Malawi is usually 90 per cent (J. A. Lawrence and others, in preparation). Twenty per cent of the animals had no record of vaccination. There was no statistically significant difference in mortality between the vaccinated and unvaccinated animals, by the χ^2 test (Table 1).

The immune status of about 20 animals from each group was assessed by serological examination using indirect immunofluorescence on August 27, 1991 (Table 2). There was no statistically significant difference in the prevalence of antibodies between the groups, suggesting that the supposedly unvaccinated animals had been exposed at some time to a high level of natural challenge or had in fact been vaccinated and not recorded. The animal that died

J. A. Lawrence, BSc, DPhil, DTVM, MRCVS, **J. Malika,** BVM, MSc, **A. P. Whiteland,** MIScT, RegSciT, **P. Kafuwa,** DipLabTech, Project GCP/RAF/259/DEN, Production of Anaplasma, Babesia and Heartwater Vaccines for East and Central Africa, PO Box 30750, Lilongwe 3, Malawi