

Electronic Supplementary information

Elevated serum antibody responses to synthetic mycobacterial lipid antigens among UK farmers: an indication of exposure to environmental mycobacteria?

Table S1: Study demographics of participants with measured antigen data

Set	Group	n	Male	Female	Median age
First study	WA	35	32	3	31
First study	WF	45	39	6	49
First study	WR	19	4	15	44
First study	WU	31	14	17	41
First study	SA	20	20	0	49
First study	SF	85	70	156	53
First study	SR	48	32	16	51
First study	SU	95	78	176	49
Second study	Farmer	76	73	3	50
Second study	Non-farmer	164	109	54	22

Table S2: Total responses lying above twice the median of the cohort, ranked in order: Groups: Welsh farmer (WF); urban (WU); rural (WR); abattoir (WA). Pink boxes represent values above the WHO TB- median, red boxes for those above the WHO TB+ median. * WHO TB+/- medians for n4 are based on a reduced sample set,²⁶ and excluded from total calculation.

Antigen	n3	n28	n32	n1	n39	n4*	Total (excluding n4)	
WHO TB+ median ²⁶	3.40	3.03	2.83	3.24	3.36	2.58	15.86	
WHO TB- median ²⁶	0.90	0.72	0.70	0.68	0.55	1.36	3.55	
Cohort median	0.54	0.47	0.30	0.48	0.57	0.49	2.36	
Sample group								Age
WF	2.93	2.33	2.76	3.07	3.18	3.16	14.27	74
WF	3.64	1.34	1.31	2.91	3.81	1.67	13.00	55
WF	2.62	2.78	1.01	2.46	3.13	2.07	11.99	70
WF	2.08	0.92	1.35	2.23	3.05	1.49	9.63	70
WF	2.53	2.46	0.99	1.44	2.03	1.14	9.44	65
WF	2.45	1.71	1.17	0.98	2.97	1.65	9.29	65
WU	2.64	0.81	0.70	1.51	1.97	1.23	7.62	65
WF	2.10	1.32	0.65	1.18	2.10	1.14	7.36	48
WR	1.92	1.45	0.68	1.88	1.33	1.27	7.25	60
WU	1.51	0.92	0.66	1.25	2.21	1.04	6.55	41

WA	1.65	1.07	0.64	1.79	1.12	1.06	6.26	30
WF	1.59	0.60	2.36	0.74	0.90	3.10	6.20	59
WU	1.28	0.75	0.53	0.73	1.36	1.29	5.93	35
WU	0.47	0.88	0.46	1.93	2.09	0.96	5.84	28
WF	1.80	0.20	1.13	0.76	1.89	1.07	5.78	56
WF	1.44	0.69	0.65	1.13	1.74	0.91	5.65	49
WU	1.20	0.97	0.58	0.81	1.73	1.03	5.28	25
WU	1.01	0.38	0.41	0.70	1.78	0.53	4.81	62
WA	1.26	0.74	0.45	1.36	0.99	0.71	4.79	46
WU	1.33	0.70	0.46	0.65	1.60	1.63	4.75	21
WA	1.33	0.56	0.42	1.24	1.18	1.29	4.73	24

Table S3: Total responses lying above twice the mean of the whole set of Scottish samples, ranked in order: Groups: Scottish farmer (SF); Scottish urban (SU); Scottish rural (SR); Scottish abattoir (SA). Pink boxes represent above the WHO TB- median, red boxes for those above the WHO + median. * WHO TB+/- medians for n4 are based on a reduced sample set,²⁶ and excluded from total calculation.

Code	Age	n3	n28	n32	n1	n39	n4*	Total
								Excluding n4
WHO TB+ median ²⁶		3.40	3.03	2.83	3.24	3.36	2.58	15.86
WHO TB- median ²⁶		0.90	0.72	0.70	0.68	0.55	1.36	3.55
Cohort median		0.32	0.30	0.29	0.38	0.33	0.34	1.62
SA	50	4.78	3.75	3.05	3.25	4.58	2.44	19.40
SF	16	2.43	3.54	5.89	4.97	2.44	5.72	19.26
SA	54	3.52	3.10	1.58	3.68	3.11	1.86	15.00
SU	32	2.99	2.68	2.58	2.48	2.79	2.69	13.51
SR	43	2.56	2.78	1.34	2.38	1.96	1.74	11.01
SR	75	2.15	2.56	1.42	2.66	2.23	1.60	11.01
SR	62	2.53	2.12	0.90	1.82	2.57	1.39	9.94
SU	53	1.60	1.76	2.44	2.26	1.65	2.52	9.69
SU	54	2.10	1.74	1.14	1.89	2.26	1.44	9.12
SA	39	1.68	1.70	1.12	2.03	2.11	1.06	8.63
SU	58	1.90	1.68	1.12	1.56	1.90	1.34	8.15
SF	43	1.44	1.54	1.07	1.57	1.32	1.11	6.94
SF	66	1.38	1.21	0.71	1.72	1.41	0.80	6.43
SA	79	1.58	1.23	1.01	1.11	1.23	1.05	6.15
SF	53	1.18	1.08	2.20	0.66	0.98	2.27	6.12
SA	52	1.31	1.44	0.64	1.54	1.09	1.18	6.03
SR	46	1.16	1.41	0.47	1.72	1.16	0.86	5.92
SR	64	1.31	1.26	0.50	1.37	1.40	1.14	5.84
SF	47	0.99	1.27	0.77	1.52	1.15	0.99	5.71

SF	75	1.08	1.00	0.72	1.09	1.04	0.66	4.92
SA	57	1.03	0.87	0.72	1.10	0.99	1.03	4.71
SU	56	0.61	1.70	0.15	1.55	0.69	0.31	4.70
SF	64	0.53	0.63	2.08	0.61	0.63	2.87	4.48
SA	49	0.97	0.83	0.80	0.94	0.86	0.81	4.40
SA	55	0.86	0.70	1.10	1.00	0.73	1.06	4.39
SA	59	0.91	0.64	0.92	0.94	0.87	0.85	4.29
SF	53	0.97	0.89	0.68	1.06	0.68	0.49	4.27
SU	63	1.09	0.84	0.68	0.56	1.04	0.80	4.21
SA	50	0.61	0.57	1.12	1.22	0.62	1.02	4.14
SF	40	0.78	0.93	0.56	0.92	0.83	0.72	4.02
SR	54	0.40	0.35	1.01	1.33	0.88	1.29	3.98
SF	68	1.05	0.54	0.68	0.80	0.87	0.67	3.94
SA	24	0.72	0.92	0.45	1.08	0.77	0.60	3.94
SF	46	1.00	0.70	0.43	0.71	1.01	0.55	3.85
SA	54	1.06	0.58	0.66	0.80	0.70	0.62	3.79
SA	39	0.69	0.54	0.89	0.80	0.82	0.84	3.74
SF	66	0.46	0.98	0.43	1.28	0.59	0.48	3.74
SR	45	0.72	0.66	0.64	0.87	0.76	0.67	3.66
SF	56	0.74	0.70	0.57	0.84	0.80	0.62	3.66
SF	40	0.80	0.67	0.59	0.83	0.76	0.58	3.64
SA	62	0.84	0.66	1.14	0.38	0.60	1.46	3.62
SA	53	0.49	0.40	1.38	0.84	0.44	1.59	3.55
SR	39	0.64	0.95	0.45	0.73	0.75	0.48	3.52
SA	42	0.66	0.54	0.76	0.89	0.57	0.72	3.41
SA	54	0.66	0.63	0.93	0.62	0.52	0.97	3.37
SF	54	0.71	0.66	0.50	0.78	0.69	0.58	3.33

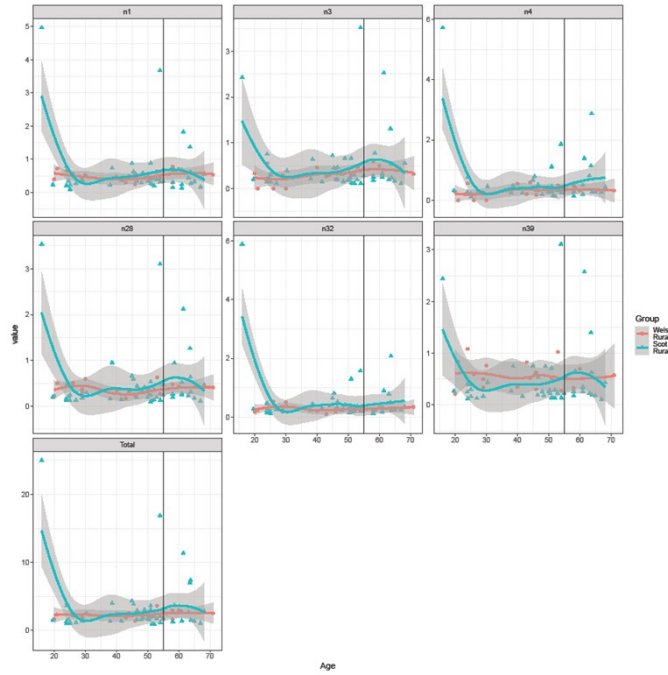
Table S4: Significance of increase in median ELISA response of first set of farmers by age bands for each antigen

			p-value		p-value
Welsh farmer	Under 55/ over 55	n1	0.11620	Under 60/over 60	0.04590
		n2	0.01870		0.04475
		n3	0.01420		0.05020
		n4	0.01795		0.06850
		n28	0.23885		0.09050
		n32	0.00645		0.05890
		n39	0.03930		0.38900
		Total	0.02490		0.51470
Scottish farmer	Under 55/over 55	n1	0.79620	Under 60/over 60	0.26015
		n2	0.29105		0.07650
		n3	0.28630		0.28730
		n4	0.13655		0.28365
		n28	0.90755		0.47545
		n32	0.19270		0.45345
		n39	0.46595		0.19045

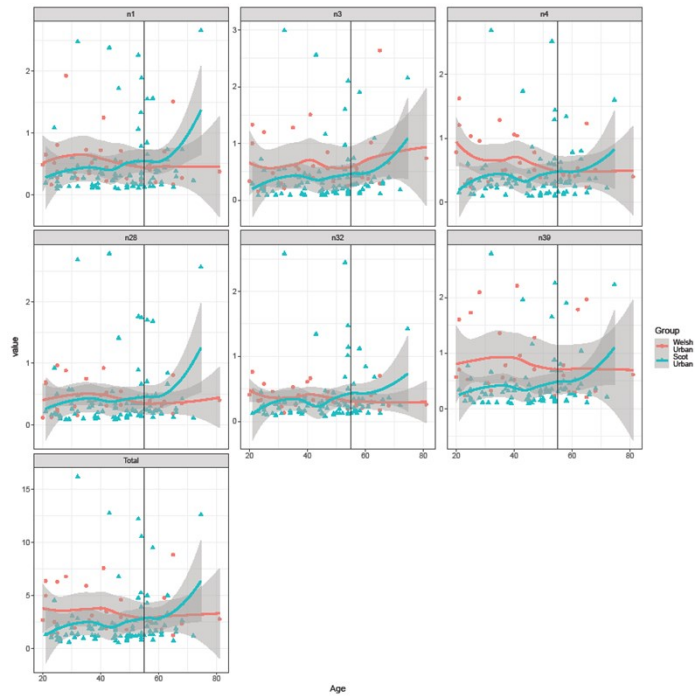
		Total	0.28515		0.39465
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Figure S4: ELISA responses for other Welsh and Scottish groups analysed by age in years with median trend lines

Rural



Urban



Abattoir

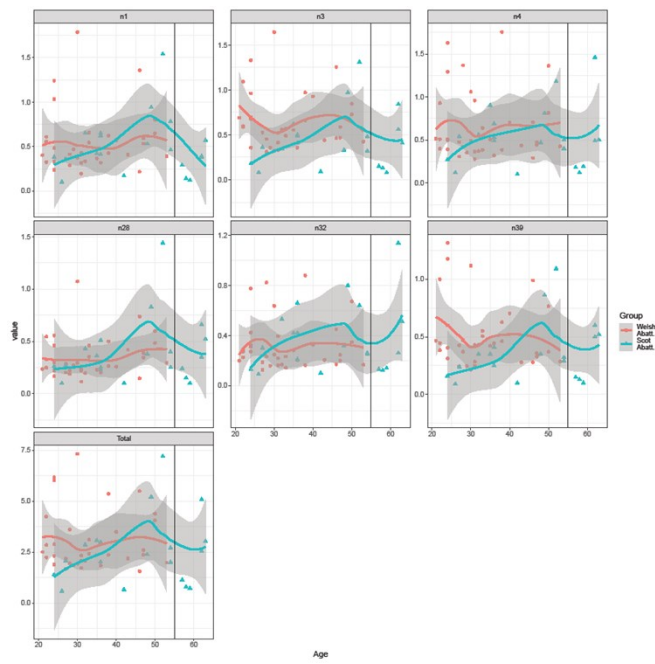


Table S5: Second set of Welsh samples above twice the median of the cohort, ranked by total assay response. Responses in pink are above the WHO TB- median for the set, those in red above the TB+ median (F: farmer; NF: non-farmer). The method was slightly different from that used for the first study (see Methods section).* WHO TB+/- medians for n4 are based on a reduced sample set,²⁶ and excluded from total calculation.

Antigen	n3	n28	n32	n39	n4*	Total excluding n4		
WHO TB+ median ²⁶	3.40	3.03	2.83	3.36	2.58	12.62		
WHO TB- median ²⁶	0.90	0.72	0.70	0.55	1.36	2.87		
Cohort median	0.22	0.17	0.22	0.23	0.31	0.91	Age	BCG
NF	5.52	5.15	3.60	5.30	5.09	19.58	29	Yes
F	3.78	3.89	2.33	4.10	3.12	14.10	69	Unknown
F	3.56	4.03	2.92	3.23	2.89	13.74	55	No
F	2.74	2.82	1.65	3.53	1.82	10.73	54	Yes
NF	2.26	2.21	2.52	2.15	1.87	9.14	43	No
NF	1.93	1.94	1.30	1.95	2.33	7.12	32	Yes
F	1.33	1.25	1.65	1.83	2.85	6.05	77	No
NF	1.59	1.79	1.16	1.44	1.47	5.98	50	Yes
F	1.85	1.65	0.95	1.45	1.51	5.89	41	Yes
F	1.11	1.45	1.63	1.68	1.25	5.87	70	No
F	1.28	1.45	1.29	1.76	2.24	5.78	57	Yes
NF	1.87	0.96	0.95	1.80	1.67	5.58	26	No
NF	1.73	0.98	0.89	1.47	1.27	5.07	56	Unknown
NF	1.03	0.87	1.35	1.41	1.47	4.66	20	Yes
F	0.83	0.91	1.30	0.86	1.17	3.90	66	No
NF	0.74	0.79	1.07	1.28	0.70	3.88	20	Yes
F	0.97	0.96	0.78	1.10	0.88	3.81	49	Yes
NF	1.16	0.76	0.46	1.39	1.69	3.77	20	No
F	1.14	1.02	0.60	0.88	0.72	3.63	52	Yes
NF	0.68	0.73	0.94	1.28	0.65	3.63	19	No
F	0.51	0.72	1.64	0.60	1.11	3.47	49	Yes
F	0.97	0.61	0.59	1.05	0.71	3.22	35	No
NF	0.99	0.47	0.63	1.00	0.85	3.10	33	Yes
NF	0.60	0.98	0.57	0.89	0.99	3.04	NA	Yes
F	0.63	0.46	1.17	0.73	1.24	2.99	77	No
F	0.55	0.86	0.89	0.67	1.43	2.97	69	Yes
NF	0.94	0.11	0.65	1.25	1.30	2.95	25	Yes
NF	0.76	0.27	1.10	0.77	1.50	2.89	40	Yes
NF	0.70	0.89	0.55	0.74	1.27	2.87	29	Yes
NF	0.36	0.54	1.50	0.42	1.74	2.81	25	Yes
NF	0.70	0.80	0.39	0.90	0.46	2.79	59	Yes
NF	0.63	0.46	0.78	0.90	0.88	2.76	22	No
NF	0.86	0.67	0.34	0.87	0.38	2.74	26	Unknown
F	0.93	0.64	0.35	0.75	0.49	2.68	50	Yes
NF	0.86	0.40	0.46	0.82	0.61	2.54	48	Yes
F	0.73	0.66	0.35	0.77	0.51	2.51	44	Yes

NF	0.57	0.51	0.67	0.76	0.54	2.50	34	Yes
F	0.44	0.58	0.93	0.45	1.13	2.41	57	Yes
F	0.80	0.36	0.43	0.79	0.61	2.38	28	Yes
NF	0.82	0.49	0.26	0.80	0.79	2.38	31	Unknown
F	0.59	0.70	0.34	0.73	0.42	2.36	49	Yes
F	0.78	0.20	0.36	0.94	0.54	2.29	28	Yes
NF	0.64	0.47	0.63	0.52	0.97	2.26	38	No
F	0.50	0.51	0.56	0.56	0.63	2.13	33	Yes
F	0.35	0.45	0.95	0.35	1.00	2.09	53	Yes
NF	0.50	0.63	0.30	0.65	0.38	2.07	40	Yes
F	0.49	0.58	0.31	0.69	0.39	2.05	60	No
NF	0.66	0.41	0.35	0.61	0.45	2.03	26	Yes
NF	0.42	0.64	0.36	0.55	0.46	1.97	24	Yes
NF	0.32	0.63	0.42	0.50	0.17	1.88	34	Yes
NF	0.48	0.43	0.59	0.33	0.84	1.84	39	Yes

Figure S6: Responses of second set of Welsh farmer and non-farmer samples analysed by age in years with median trend lines

