Epidemiol. Infect. (1996), **117**, 69–77 Copyright © 1996 Cambridge University Press

World Health Organisation – supervised interlaboratory comparison of ELISAs for the serological detection of *Salmonella enterica* serotype Enteritidis in chickens

P. A. BARROW[1], M. DESMIDT[2], R. DUCATELLE[2], M. GUITTET[3], H. M. J. F. VAN DER HEIJDEN[4], P. S. HOLT[5], J. H. J. HUIS IN'T VELT[6], P. McDONOUGH,[7] K. V. NAGARAJA[8], R. E. PORTER[9], K. PROUX[3], F. SISAK[10], C. STAAK[11], G. STEINBACH[12], C. J. THORNS[13], C. WRAY[13] AND F. VAN ZIJDERVELD[14]

[1] *Institute for Animal Health, Compton Laboratory, Compton, Nr. Newbury, Berkshire, RG20 7NN, UK*
[2] *Faculty of Veterinary Medicine, University of Gent, Casinoplein 24, B-9000, Gent Belgium*
[3] *Laboratoire Central de Recherches Avicole et Porcine, Centre Nationale d'Etudes Veterinaires et Alimentaires (CNEVA), Beaucemaine, BP53, 22440 Ploufragan, France*
[4] *Stichting Gezondheidsdienst voor Dieren in Zuid Nederland, Postbus 4, 5280 AA Boxtel The Netherlands*
[5] *USDA Southeast Poultry Research Laboratory, PO Box 5657, Athens, Georgia 30604, USA*
[6] *Department of the Science of Food of Animal Origin, Faculty of Veterinary Medicine, Utrecht University, The Netherlands*
[7] *Diagnostic Laboratory, New York State College of Veterinary Medicine, Cornell University, PO Box 5786, Ithaca, New York 14853, USA*
[8] *Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Minnesota, 1971 Commonwealth Avenue, St Paul, Minnesota 55108, USA*
[9] *Animal Disease Diagnostic Laboratory, Purdue University, 1175 VADL, West Lafayette, Indiana 47907-1175, USA*
[10] *Veterinary Research Institute, Hudcova 70, 621-32 Brno, Czech Republic*
[11] *Bunderinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin, Postfach 33 00 13, D-14191, Berlin 33, Germany*
[12] *Bunderinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin, Bereich Jena, D-07722 Jena, Germany*
[13] *Central Veterinary Laboratory, New Haw, Weybridge, Surrey, KT15 3NB, UK*
[14] *Central Veterinary Institute (CD-DLO), PO Box 65, NL-8200 AB, Lelystad, The Netherlands*

(*Accepted 27 January 1996*)

SUMMARY

A collaborative exercise, supervised by the World Health Organisation, was set up to compare ELISAs used for the serological detection of *Salmonella enteritica* serotype Enteritidis in chickens. The aim was to ascertain how far agreement could be reached on the interpretation of optical density readings for high titre, intermediate titre and low titre sera. Two sets of sera were sent to 14 participants. The first set compared high, medium and low titre sera raised in specified-pathogen-free and commercial broiler breeder chickens. The second set comprised 20 sera of different antibody titres raised in commercial birds reared under laboratory conditions and sent blind. Both indirect and double-antibody sandwich blocking ELISAs were used with a number of different detecting antigens. With a few exceptions good agreement was reached on the interpretation of results obtained from high and low titre sera from the optical density obtained with a single serum dilution. Differences were observed in the interpretation of medium titre sera. The results suggested that most ELISAs produce reasonably comparable results and that practical problems may arise from interpretation of the results mainly as a result of the choice of the criteria used for differentiating sera obtained from infected and uninfected chickens. These problems are discussed.

https://doi.org/10.1017/S095026880000114X Published online by Cambridge University Press

INTRODUCTION

In many countries *Salmonella enterica* serotype Enteritidis has replaced *S. Typhimurium* as the predominant serotype in both human disease and in poultry [1]. Bacteriological methods are generally required by national and international legislation for monitoring procedures to provide epidemiological evidence from which control measures might be devised [2, 3]. The desire for detailed and rapidly obtainable information on the infection status of flocks has led to the independent development in several countries of ELISAs for the detection of circulating specific IgG whose production is induced by invasive serotypes such as *S.* Enteritidis, *S.* Typhimurium, *S.* Gallinarum, *S.* Pullorum and *S. arizonae* (for review see [4]). The advantages of the ELISA over other serological assays in coping with large numbers of samples of either serum or egg yolk together with miniaturization, mechanization and the availability of high quality reagents has led to the extensive use of this system.

European Union legislation [3] allows for the use of serological methods for screening purposes provided that the method chosen is able to provide similar guarantees of success produced by hatchery investigations. Bacteriological confirmation of infection is required nevertheless. ELISAs are already in use in a number of countries for this purpose, particularly in the Netherlands, where they are an integral part of the *S.* Enteritidis control programme, and in the UK. In such countries ELISAs have generated a great deal of useful information on infection frequencies within and between flocks.

As a result of successful standardisation of ELISA procedures for brucellosis [5, 6], the World Health Organisation (WHO) recognized the value of such assays in monitoring salmonella infections in both poultry and cattle. Since different ELISAs had already been developed, largely independently, it was suggested at recent meetings of the WHO working group on salmonella immunization in animals that a standardization process should be instigated, at the very least to enable different groups working in this area to agree on the interpretation of results obtained by assaying standard sera. An interlaboratory trial was therefore set up to assess the performance of different ELISAs using a bank of sera of known antibody status raised in chickens against *S.* Enteritidis. The aim was not to compare such assays with other procedures such as immunoblotting or microantiglobulin [4] but rather to assess whether standardization procedures might be necessary and if so how this might be done.

MATERIALS AND METHODS

Trial laboratories

A number of laboratories were contacted which had developed assays and were using them either for research purposes or for screening poultry flocks for invasive *Salmonella* serotypes.

ELISAs

Amongst the different laboratories taking part two main types of assay were used, details of which have been published elsewhere (see below). Although details will not be presented in detail here, a summary of the stages of the different ELISAs is shown in Table 1. An indirect ELISA, comprising antigen-coated plates, for the detection of IgG in serum or yolk was used by a number of groups. A variety of detecting antigens have been used including lipopolysaccharide LPS [7–9], whole flagella [10], recombinant flagellin protein containing the serotype specific flagellin fragment [11], SEF14 fimbrial antigen [12], outer membrane proteins [13] and disrupted whole bacterial cell proteins [7]. In the present study several participants' assays used LPS preparations. Flagella antigen was used by participants C.S., J.H.V., P.M.D., P.H./R.P. and F.V.Z., outer membrane protein by F.S. and K.N. and SEF14 fimbrial antigen by C.T. In addition, G.S. used whole-bacterial-cell protein antigen, repeating some of the tests with LPS. The other basic method used, the double antibody sandwich (DAS), ELISA, uses plates coated with monoclonal antibody, followed by a pure or crude antigen preparation. Test samples are then applied either followed by a conjugate (CT) or by a conjugated monoclonal antibody which will not bind if the sample contains specific antibodies [14]. Various preparations, such as 1M mineral acid, 0·5 M NaOH or 1/20–1/50 diluted dishwashing detergent, were used to stop the reaction. In some cases nothing was used and the plate was read immediately.

Sera

Two sets of sera were used.

The first set was prepared by oral inoculation of a group of five 3-week-old specified-pathogen-free

https://doi.org/10.1017/S095026880000114X Published online by Cambridge University Press

Table 1. *Summary of procedures used for ELISAs by different laboratories*

	Participants													
	C.W.	H.v.H.	M.G.	P.B.	R.D.	G.S.	C.S.	J.H.V.	P.M.D.	P.H./R.P.	F.S.	K.N.	F.V.Z.	C.T.
Type of ELISA	Indirect	Indirect	Indirect	Indirect	Indirect	Indirect	Indirect	Indirect	Indirect	Indirect	Indirect	Indirect	DAS	DAS
Type of antigen	LPS	LPS	LPS	LPS	LPS	WCP/LPS	Fla	Fla	Fla	Fla	OMP	OMP	Fla	SEF14
Type of plate	PVC (Falcon)	PStyr (Costar)	PStyr (Costar)	PVC (Dynatech)	PStyr (Nunc)	PStyr (Plastic GmbH)	PStyr (Greiner)	PStyr (not known)	PStyr (Nunc)	PStyr (Dynatech)	PStyr (GAMA)	PStyr (Dynatech)	PStyr (Greiner)	PStyr (Nunc)
Coating conditions	pH 9·6 1 h 37 °C	pH 9·6 14 h 20 °C	pH 9·8 2 h 37 °C	pH 9·6 1 h 37 °C	pH 9·6 24 h 4 °C	pH 9·6 18 h 20 °C	pH 9·6 18 h 20 °C	pH 9·6 18 h 4 °C	pH 9·6 3 h 37	pH 9·6 18 h °C	pH 9·6 4 h 37 °C	pH 9·6 24 h 4 °C	pH 9·6 18 h 37 °C	pH 9·6 18 h 4 °C
Washing conditions	DW	PBST	PBST	PBST	PBST	PBST	PBST	PBST	PBST	PBST	PBST	PBST	DW + Tween	PBST
Conjugate (αchIgG)	G-AP (ICN)	R-HRP	R-AP (Sigma)	R-AP (Sigma)	R-HRP (Nordic)	R-HRP (Nordic)	R-HRP (Nordic)	R-HRP (Nordic)	R-HRP	M-chIgG G-mouse IgG-Ap	P-HRP	G-HRP (Kirkegaard & Perry	mAb-Fla - HRP	R-Ap
Substrate (& conc.)	PNP (1 mg/ml)	TMB (1 mg/ml)	PNP (1 mg/ml)	PNP (1 mg/ml)	OPD (0·7 mg/ml) H_2O_2 (2·2 mg/ml)	ABTS (0·4 mg/ml) H_2O_2 (0·25 mg/ml)	ABTS (0·4 mg/ml) H_2O_2 (0·25 mg/ml)	ABTS (1 mg/ml) H_2O_2 (0·15 mg/ml)	ABTS H_2O_2	PNP (5 mg/ml)	TMB (0·1 mg/ml) H_2O_2 (60 μg/ml)	OPD (0·18 mg/ml) H_2O_2 (34 μg/ml)	PAS (1 mg/ml) H_2O_2 (50 μg/ml)	PNP
Stop		H_2SO_4		NaOH	HCl	Deterg.	Deterg.			NaOH		H_2SO_4		NaOH
λ (nm)	405	450	405/490	405	492	405	405	405		405	490	490	450	405

The participants were as follows: C.W., C. Wray; H.v.H., H. van der Heijden; M.G., M. Guittet; P.B., P. Barrow; R.D., R. Ducatelle; G.S., G. Steinbach; C.S., C. Staak; J.H.V., J. Huis in't Veld; P.M.D., P. MacDonough; P.H./R.P., P. Holt/R. Porter; F.S., F. Sisak; K.N., K. Nagaraia; F.V.Z., F. van Zijderveld; C.T., C. Thorns. Antigens were lipopolysaccharide (LPS), whole cell protein (WCP), flagella (Fla), outer membrane protein (OMP) or SEF14 fimbrial antigen (SEF14). Plates were of polyvinyl chloride (PVC) or polystyrene (PStyr). Washing was with distilled water (DW) or phosphate buffered saline with Tween 20 (PBST). Conjugates were prepared in goat (G-), rabbit (R-), mouse (M-), or pig (P-) and were linked to horse-radish peroxidase (HRP) or alkaline phosphatase (AP). FVZ used mouse monoclonal antibody (mAb-). Substrates were ABTS (2,2′azinobis (3-ethyl benzthiazolone sulphonic acid)), PAS (*p*-amino salicylic acid), PNP (*p*-nitrophenyl phosphate), OPD (*O*-phenylene diamine) and TMB (3,3′,5,5′-tetramethyl benzidene dihydrochloride).

https://doi.org/10.1017/S095026880000114X Published online by Cambridge University Press

(SPF) Light Sussex chickens, housed at the Institute for Animal Health, Compton, UK, under conditions described previously [15] with 0·3 ml of a 10 ml nutrient broth culture of *S.* Enteritidis P125589 (phage type 4, [9]) which had been incubated for 18 h at 37 °C with shaking and which contained *c.* 10^9 cfu/ml. These chickens were then placed in the same pen as 25 uninfected SPF chickens. All the chickens were bled every week for 7 weeks and then every second week until sufficient sera had been obtained.

This set also included sera obtained from a commercial company which had screened sera from customers, some of whose birds had shown bacteriological evidence of *S.* Enteritidis infection.

The second set was obtained by hatching commercial broiler breeder eggs from a flock free of *S.* Enteritidis, *S.* Typhimurium, *S.* Gallinarum and *S.* Pullorum. They were hatched and reared under SPF conditions using commercial food. They were infected and bled in the same way as the first group of Light Sussex chickens.

Sera were titrated using an indirect ELISA with *S.* Enteritidis LPS as antigen [7] and were stored frozen. They were sent to the participants containing either 0·02% w/v sodium azide (first set) or 0·02% w/v sodium merthiolate (second set) as preservative.

Protocol of trial

The trial comprised two stages. In the first, 3 strong positive, 3 weak positive and 3 negative sera from SPF chickens were distributed to participants. In addition, 2 strong positive and 3 negative sera obtained from commercial chickens were sent, in view of reports that sera from uninfected commercial chickens give higher titres than SPF birds [10, 16]. The sera were tested in the participants' laboratories using their own ELISA, run in triplicate on 3 different days (9 times in total). The results were reported as 9 individual optical density values (OD) with the blank already subtracted, together with the mean and standard deviation and the OD values of the positive and negative control sera in routine use.

In the second stage 20 chicken sera were sent to all laboratories and run blind in triplicate. The results and their interpretation (positive or negative) of the results were then returned.

Bacteriological data were not collected since this was not a field trial to assess the sensitivity of assays in comparison with culture.

RESULTS

Stage one

The results presented in Table 2 are the arithmetic mean of the nine results from each laboratory. In addition, the optical density values obtained for the standard positive and negative sera are shown together with the standard deviation. It must be noted that competitive blocking ELISAs (column FVZ) give low OD values for high titre sera and high values for low titre sera.

In general, most of the assays behaved in a similar manner such that sera identified as high titre gave high OD values and medium and low titre sera gave intermediate and low values. Three participants (H.V.H., R.D. and F.V.Z.) found that the medium titre sera gave similar values to the high titre sera. This was also the case with K.N. who also recorded high values with the commercial low titre sera, higher than the values obtained by P.M.D. and C.S. for the higher titre sera. Whether this relates to the use by C.S. and P.M.D. of flagellar antigen is unclear. G.S. found that one of the commercial low titre sera produced a high OD with a sonicated salmonella antigen. Other results supplied by this participant showed that this was eliminated by using LPS as detecting antigen.

The positive control sera used by the participants produced high OD values with two exceptions (M.G., P.M.D.) and the negative control sera gave very low readings with one exception (K.N.). There was considerable variation in the standard deviations obtained by the different participants with greater variation seen with the assays of P.B., C.W. and F.S. and negligible variation seen with the assay of P.M.D.

Stage two

Although the sera were sent blind they have been arranged in Table 3 in order of decreasing titre. With the exception of the KN and CT assays the extent of correlation between the results produced by the different participants was again high. There was a smaller gradation between high and low OD values in one set of results (KN) and one participant (HVH) produced much higher values generally although a strong gradation from high to low titres was demonstrated. This participant was using assay conditions for optimum sensitivity.

The optical densities from Table 3 were interpreted by the participants and these are shown in Table 4.

https://doi.org/10.1017/S095026880000114X Published online by Cambridge University Press

Table 2. *ELISA absorbance values obtained with standard sera of known titre using participants' own ELISA for* Salmonella *Enteritidis*

Serum sampled		Titre against LPS	Absorbance obtained by the following participants												
			C.W.	H.v.H.	M.G.	P.B.	R.D.	G.S.	C.S.	P.M.D.	P.H./R.P.	F.S.	K.N.	F.V.Z.	C.T.
			Indirect ELISA using, as antigen											DAS ELISA using	
			LPS	LPS	LPS	LPS	LPS	WCP	Fla	Fla	Fla	OMP	OMP	Fla	SE14
SPF															
High	1	1:102400	1·21	2·26	1·37	2·81	2·26	1·18	0·79	0·48	1·67	0·99	1·57	0·01	2·15
	2	1:51200	1·22	2·26	1·38	2·62	2·22	1·29	0·80	0·46	1·45	0·96	1·56	−0·01	0·83
	3	1:51200	1·23	2·25	1·49	2·73	2·31	1·25	0·78	0·46	1·48	0·95	1·59	−0·01	1·41
Medium	1	1:3200	0·52	2·24	0·51	0·64	1·80	0·58	0·38	0·29	0·63	0·38	1·52	−0·01	0·57
	2	1:6400	0·70	2·20	0·71	0·75	1·84	0·23	0·38	0·31	0·69	0·25	1·54	−0·01	0·34
	3	1:12800	0·94	2·22	0·86	1·08	2·27	0·25	0·32	0·36	0·72	0·27	1·55	−0·01	0·33
Low	1	1:200	0·04	0·05	0·00	0·08	0·01	0·08	0·10	0·00	0·01	0·03	0·21	0·94	0·15
	2	1:200	0·02	0·07	0·01	0·09	0·01	0·10	0·10	0·01	0·02	0·02	0·41	0·96	0·22
	3	1:200	0·02	0·07	0·01	0·08	0·03	0·13	0·11	0·01	0·01	0·02	0·39	0·93	0·21
Commercial															
High	1	1:51200	0·75	2·26	0·90	1·08	2·42	1·13	0·69	0·44	1·25	0·87	1·56	0·01	2·05
	2	1:25600	0·83	2·26	0·86	1·46	2·14	1·09	0·61	0·40	1·11	0·81	1·58	0·02	1·66
Commercial															
Low	1	1:400	0·09	0·17	0·01	0·14	0·08	0·08	0·13	0·02	0·18	0·00	0·92	1·03	0·79
	2	1:400	0·13	0·16	0·00	0·14	0·06	0·12	0·15	0·03	0·27	0·00	0·79	0·99	0·87
	3	1:800	0·09	0·06	0·02	0·12	0·02	1·08	0·34	0·04	0·34	0·35	–	0·90	0·46
Positive control		1:25600	1·46	1·49	0·60	1·54	1·69	1·61	1·00	0·31	0·98	ND	1·53	0·00	3·50
Negative control		1:200	0·03	0·18	0·00	0·09	0·00	0·09	ND	0·03	0·02	ND	0·38	0·96	0·10
Standard deviations of test sera		High titre sera	0·23–0·16	0·12–0·09	0·13–0·05	0·26–0·10	0·10–0·02	0·08–0·04	0·08–0·02	0·01–0·00	0·11–0·02	0·42–0·20	0·08–0·04	0·02–0·00	0·18
		Low titre sera	0·08–0·01	0·11–0·02	0·02–0·01	0·06–0·02	0·01	0·03–0·01	0·05–0·01	0·01–0·00	0·07–0·00	0·05–0·02	0·21–0·05	0·07–0·04	0·03

LPS, lipopolysaccharide; Fla, flagella protein; OMP, outer membrane protein, SEF14, SEF14 fimbrial antigen; WCP, whole cell protein; ND, no value given; DAS ELISA, double antibody sandwich ELISA. For key participants see Table 1.

https://doi.org/10.1017/S095026880000114X Published online by Cambridge University Press

Table 3. *ELISA absorbance values obtained with 20 sera sent blind using participants's own ELISA for* Salmonella *Enteritidis*

		Absorbances obtained by the following participants												
		C.W.	H.v.H.	M.G.	P.B.	R.D.	G.S.	C.S.*	J.H.V.	P.H./R.P.	F.S.†	K.N.	F.V.Z.	C.T.
	Titre	Indirect ELISA using, as antigen											DAS ELISA using	
Serum no.	against LPS	LPS	LPS	LPS	LPS	LPS	WCP	Fla	Fla	Fla	OMP	OMP	Fla	SEF14
A	51200	1·62	3·12	1·70	1·69	2·30	1·61	7	1·27	1·03	P	0·95	0·011	0·30
B	51200	1·24	3·17	1·37	1·36	2·20	1·44	6·5	0·52	1·01	P	0·98	−0·04	0·26
C	25600	1·37	3·12	1·23	1·43	2·26	1·55	7	1·22	1·16	P	0·97	0·02	1·39
D	6400	1·14	3·01	1·66	1·14	2·21	1·44	6·5	1·65	1·03	P	0·87	0·02	0·68
E	6400	1·14	3·08	1·36	1·22	2·12	1·47	6·5	1·60	1·34	P	0·81	−0·00	0·34
F	6400	1·49	3·09	1·42	1·83	2·12	1·48	7	1·46	1·15	P	0·74	0·05	2·32
G	6400	0·62	2·88	0·75	0·52	1·91	1·53	7	0·41	0·58	P	0·91	−0·00	0·15
H	3200	0·95	3·98	1·14	0·83	1·55	1·23	3·5	1·42	0·89	P	0·77	−0·00	0·43
I	1600	0·43	2·65	0·71	0·35	0·91	0·80	3	0·25	0·50	P	1·07	0·08	0·36
J	1600	0·14	3·08	0·31	0·14	0·36	0·40	3·5	0·20	0·21	P	0·70	0·07	0·27
K	1600	0·29	3·09	0·46	0·23	0·67	0·67	2	0·21	0·28	D	0·74	0·11	0·69
L	1600	0·13	0·73	0·19	0·17	0·65	0·64	1·5	0·23	0·22	N	0·34	0·10	2·31
M	1600	0·06	1·36	0·32	0·10	0·19	0·53	1·5	0·39	0·31	N	0·38	0·07	2·39
N	800	0·13	2·44	0·31	0·15	0·20	0·74	1·5	0·23	0·41	D	0·64	0·01	1·15
O	800	0·11	1·58	0·31	0·49	0·29	0·37	3·5	0·45	0·05	D	1·08	0·05	0·87
P	800	0·08	1·16	0·26	0·33	0·16	0·36	3	0·38	0·08	N	0·40	0·08	1·30
Q	800	0·05	0·74	0·12	0·09	0·06	0·16	2	0·14	0·39	N	0·66	0·39	0·75
R	400	0·04	0·99	0·14	0·05	0·07	0·20	2	0·16	0·15	N	0·35	0·15	1·46
S	400	0·05	0·35	0·09	0·03	0·07	0·11	1·5	0·12	0·46	N	0·76	0·46	0·46
T	400	0·06	0·50	0·14	0·06	0·07	0·19	0·5	0·13	0·18	N	0·63	0·18	0·36

See Table 2 for LPS, Fla, OMP, SEF14.

* C.S. provided a scale representing gradation of titres, i.e. 1 = 0–1/10, 2–6 = 1/20–1/320, 7 = > 1/640.

† Absorbance values not provided by F.S. P, positive; D, doubtful; N, negative.

For key to participants see Table 1.

https://doi.org/10.1017/S095026880000114X Published online by Cambridge University Press

Table 4. *Participants' own interpretation of the data in Table 3*

		Interpretation of absorbances by the following participants												
		C.W.	H.v.H.	M.G.	P.B.	R.D.	G.S.	C.S.	J.H.V.	P.H./R.P.	F.S.	K.N.	F.V.Z.	C.T.
	Titre	Indirect ELISA using, as antigen											DAS ELISA using	
Serum no.	against LPS	LPS	LPS	LPS	LPS	LPS	WCP	Fla	Fla	Fla	OMP		Fla	SEF14
A	51200	+	++++	+	+	+	+	++++	+++	+	+	+	+	+
B	51200	+	++++	+	+	+	+	+++	++	+	+	+	+	+
C	25600	+	++++	+	+	+	+	++++	+++	+	+	+	+	+
D	6400	+	++++	+	+	+	+	+++	++++	+	+	+	+	+
E	6400	+	++++	+	+	+	+	+++	++++	+	+	+	+	+
F	6400	+	++++	+	+	+	+	++++	+++	+	+	±	+	+
G	6400	+	++++	+	+	+	+	++++	+	±	+	+	+	−
H	3200	+	++++	+	±	+	+	++	+++	+	+	±	+	+
I	1600	+	++++	+	±	±	+	++	+	±	+	+	+	+
J	1600	−	++++	+	∓	−	±	++	+	±	+	±	+	+
K	1600	+	++++	+	∓	±	+	++	+	±	±	±	+	+
L	1600	−	+	+	∓	+	+	−	+	±	−	−	+	+
M	1600	−	+	+	−	−	±	−	+	±	−	−	+	+
N	800	−	++++	+	−	−	+	−	+	±	±	±	+	+
O	800	−	++	+	−	±	+	++	+	±	±	+	+	+
P	800	−	+	+	−	−	±	++	+	±	−	±	+	+
Q	800	−	+	−	−	−	−	±	−	−	−	±	+	+
R	400	−	+	−	−	−	±	+	−	−	−	−	+	+
S	400	−	−	−	−	−	−	−	−	−	−	±	±	+
T	400	−	±	−	−	−	±	−	−	−	−	±	+	+

See Table 2 for LPS, Fla, OMP, SEF14.
For key to participants see Table 1.

There was again no problem in interpreting the OD values of the high titre sera whereas a mixture of positive, weak positive and negative interpretations were obtained with the intermediate titre group. Most of the negatives were found in the low titre group. The interpretations of four participants (P.B., R.D., F.S. and C.W.) were very similar. Some participants (M.G., K.N., G.S., C.T., F.V.Z.) interpreted at least one low titre serum with a value as high as some of the highest titre sera.

DISCUSSION

The results from the first part of this comparative evaluation showed that, by and large, a good correlation was obtainable between the OD values and IgG titre of the sera when tested in different laboratories with different assays. A similar degree of correlation has been found in a joint exercise to compare ELISAs for the serological detection of *S.* Dublin infection in cattle, also supervised by WHO [17]. The main problems arising from this exercise lay in the high OD values obtained by some participants with the intermediate titre sera. Since these had been obtained from chickens which had been infected with *S.* Enteritidis and the IgG titres in these sera were so much higher than those of the low titre sera this problem may not be intractable.

Because of this a greater number of intermediate and low titre sera were included in the second part of the exercise. Although in a small number of cases this problem re-occurred, for low titre sera most assays produced OD values considerably lower than those for the intermediate titre sera. Despite this, a number of participants interpreted these results as positive. The extensive use of these assays in the field should be questioned until such problems have been resolved. In most cases these discrepancies of interpretation are the results of different criteria used in determining cut-off OD values above which a reading is considered positive. In practice most of these assays would therefore be expected to produce similar results and thus the major problem is one of the choice of a cut-off value. This is of considerable significance to the

https://doi.org/10.1017/S095026880000114X Published online by Cambridge University Press

practical use of ELISAs for serological screening of poultry flocks for *S.* Enteritidis and other invasive serotypes. Absolute unanimity in interpretation of low titre sera is more important than the same for high titre sera because when used as a flock test the decision to regard a flock as serologically positive may rest on a small number of sera which produce OD values just above the cut-off value. A possible way of overcoming this problem could be to use, as a criterion, the presence of individual sera in a flock sample which contain very high titre antibodies and which therefore should produce high optical densities, well above the cut-off usually obtained from sera from uninfected birds. This has been discussed at length elsewhere [18, 19]. This does not negate the value of positive control sera which should anyway be included. One interpretation of the discrepancies in the actual values is that the temporal antibody response to antigens other than LPS and flagellin, such as SEF14 and OMPs, may be different. The generation of information on these points from experimental infections would assist in the interpretation of individual assays.

One weakness of this comparative exercise is that sera were produced and titrated by one of the participants using one assay. Sera produced by another laboratory may have produced different results through, amongst other things, choice of test or antigen. This does not, however, appear to be a major problem since a number of quite different assays compared produced consistent results.

Another problem raised by a recent workshop on ELISAs for salmonella in poultry [19] was one of specificity in using LPS-based ELISAs for group B and D *Salmonella* serotypes. Mild periodate treatment of group D LPS has been shown to remove the cross-reacting 12 epitope, allowing increased specificity for *S.* Enteritidis and other serotypes such as *S.* Gallinarum and *S.* Pullorum [20]. For screening for *S.* Enteritidis only, SEF14 antigen [12] may be used, since this is found only in this and one or two other rare serotypes such as *S.* Moscow and *S.* Blegdam. Research into the use of other antigens such as flagella which are already incorporated into several of the ELISAs in this study is continuing [11]. Flagella were used because of the potentially greater antigenic diversity although cross reactions may occur. Cloning the serotype-specific fragment away from the conserved N- and C-terminal parts may allow greater specificity for a number of serotypes and enable relatively large amounts of antigen to be produced [21]. The use of combinations of detecting antigens to enhance specificity has yet to be studied in sufficient detail.

In a similar comparative exercise the WHO were able to recommend a standardized procedure for the use of ELISA for the serological detection of brucellosis in cattle and a number of other hosts including man [5, 6]. With the present assays for salmonella already in extensive use it is uncertain whether standardization can be taken to similar lengths. Rather than reach a point where an exact protocol is recommended it might be more appropriate for official testing to be carried out by assays which have been approved by standardisation against a set of sera similar to that used here. This work could be organized by an international organization such as the WHO or European Union.

REFERENCES

1. Rodrigue DC, Tauxe RV, Rowe B. International increase in *Salmonella enteritidis*: a new pandemic? Epidemiol Infect 1990; **105**: 21–7.
2. Statutory Instruments. The poultry breeding flocks and hatcheries (Registration and Testing) Order no. 1963. London: HMSO, 1989.
3. Council Directive 92/117/EEC of 17 December 1992 concerning measures against specified zoonoses and specified zoonotic agents in animals and products of animal origin in order to prevent outbreaks of food-borne infections and intoxications. Of J Europ Communities 1993; **L62**: 38–48.
4. Barrow PA, ELISAs and the serological analysis of salmonella infections in poultry: a review. Epidemiol Infect 1992; **109**: 361–9.
5. Report. Unpublished report of the World Health Organisation informal meeting on enzyme immunoassay for brucellosis management. Nepean, Ontario, Canada, 29–30 May 1991. VPH/89.84. Geneva: World Health Organisation, 1989.
6. Report. Unpublished report of the World Health Organisational informal consultation on enzyme immunoassay for brucellosis diagnosis and research. Geneva, 5–6 April 1990. VPH/90.90. Geneva: World Health Organisation, 1990.
7. Hassan JO, Barrow PA, Mockett APA, McLeod S. Antibody response to experimental *Salmonella typhimurium* infection in chickens measured by ELISA. Vet Rec 1990; **126**: 519–22.
8. Nicholas RAJ, Cullen GA. Development of an ELISA for detecting antibodies to *Salmonella enteritidis* in chicken flocks. Vet Rec 1991; **128**: 74–6.
9. Barrow PA, Lovell MA. Experimental infection of egg-laying hens with *Salmonella enteritidis*. Avian Pathol 1991; **20**: 339–52.
10. Timoney JF, Sikora N, Shivaprasad HL, Opitz M. Detection of antibody to *Salmonella enteritidis* by a gm flagellin-based ELISA. Vet Rec 1990; **127**: 168–9.

https://doi.org/10.1017/S095026880000114X Published online by Cambridge University Press

11. Baay MFD, Huis in't Veld JHJ. Alternative antigens reduce cross-reactions in an ELISA for the detection of *Salmonella enteritidis* in poultry. J Appl Bact 1993; **74**: 243–7.
12. Thorns CJ, Sojka MG, Chasey D. Detection of a novel fimbrial structure on the surface of *Salmonella enteritidis* by using a monoclonal antibody. J Clin Microbiol 1990; **28**: 2409–14.
13. Kim CJ, Nagaraja KV, Pomeroy BS. Enzyme-linked immunosorbent assay for the detection of *Salmonella enteritis* infections in chickens. Amer J Vet Res 1991; **52**: 1069–74.
14. Van Zijderveld FG, Van Zijderveld-van Bemmel AM, Anakotta J. Comparison of four different enzyme-linked immunosorbent assays for serological diagnosis of *Salmonella enteritidis* infections in experimentally infected chickens. J Clin Microbiol 1992; **30**: 2560–6.
15. Smith HW, Tucker JF. The effect of antibiotic therapy on the faecal excretion of *Salmonella typhimurium* by experimentally infected chickens. J Hyg 1975; **75**: 275–92.
16. McLeod S, Barrow PA. Lipopolysaccharide-specific IgG in egg yolk from two chicken flocks infected with *Salmonella enteritidis*. Lett Appl Microbiol 1991; **13**: 294–7.
17. Dilling GW, Smith BP. International standardisation of ELISA for serologic detection of *Salmonella dublin* carrier cows. Proceedings of the International Symposium on Salmonella and Salmonellosis. Ploufragan, France 1992; 87–8.
18. Barrow PA. Use of ELISAs for monitoring Salmonella in poultry. Vet Rec 1994; **134**: 99.
19. Barrow PA, Wray C, eds. Proceedings of the European Commission Workshop on ELISAs for serological diagnosis of Salmonella infection in poultry. Brussels, 7–9 June 1993. Directorate General for Agriculture, Commission of the European Communities, Brussels, 1994.
20. Van der Heijden HMJF. Effect of periodate oxidation of LPS on the specificity of Salmonella-ELISAs in poultry. Proceedings of the European Commission Workshop on ELISAs for serological diagnosis of Salmonella infection in poultry, Brussels, 7–9 June 1993. Directorate General for Agriculture, Commission of the European Communities, Brussels, 1994.
21. Huis in't Veld JHJ, van Asten AJAM, Baay MFD, Kusters JG, van der Zeijst BAM. Selection of alternative antigens for the development of a specific ELISA for the detection of *Salmonella enteritidis* in poultry. Proceedings of the European Commission Workshop on ELISAs for serological diagnosis of Salmonella infection in poultry, Brussels, 7–9 June 1993. Directorate General for Agriculture, Commission of the European Communities, Brussels, 1994.

https://doi.org/10.1017/S095026880000114X Published online by Cambridge University Press