Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

ELSEVIER

Available online at www.sciencedirect.com
SCIENCE DIRECT®

Preventive Veterinary Medicine 71 (2005) 91–103

PREVENTIVE VETERINARY MEDICINE

www.elsevier.com/locate/prevetmed

Evaluation of a French ELISA for the detection of *Salmonella* Enteritidis and *Salmonella* Typhimurium in flocks of laying and breeding hens

E. Jouy [a,b,*], K. Proux [b], F. Humbert [a], V. Rose [a], F. Lalande [a], C. Houdayer [b], J.-P. Picault [b], G. Salvat [a]

[a] *Department of Hygiene and Quality of Poultry and Pork Products, French Agency for Food Safety, AFSSA – Site de Ploufragan, BP 53, 22440 Ploufragan, France*

[b] *Department of Avian and Rabbit Virology, Immunology and Parasitology, French Agency for Food Safety, AFSSA – Site de Ploufragan, BP 53, 22440 Ploufragan, France*

Received 9 August 2004; received in revised form 24 May 2005; accepted 9 June 2005

Abstract

In France, the regular and compulsory detection of *Salmonella* Enteritidis (SE) and *Salmonella* Typhimurium (ST) in flocks of breeding and laying hens is based on bacteriological examination of environmental swabs and faeces samples. The aim of this study was to compare this bacteriological examination with a serological method (ELISA) developed in our laboratory. This ELISA was first evaluated by use of artificially infected hens. During these experimental infection studies, several groups of hens were inoculated with SE, ST, different vaccines and different *Salmonella* serovars to calculate the experimental parameters of our ELISA. Then, in a field study, 43 flocks were followed monthly using two bacteriological samples (environmental swab and pool of faeces) and 20 serological samples (sera or yolks). Twenty-seven flocks without SE or ST gave a negative serological response throughout their surveillance. Among the 10 various serovars different from SE and ST isolated in this study, *S*. Heidelberg, *S*. Agona and *S*. Hadar gave seropositive results in seven flocks. Consequently, this ELISA was not specific of SE and ST as it detected serovars sharing or not common antigens with SE and ST. Seropositive results were also obtained each month for two flocks where no *Salmonella* could be isolated. Finally, in seven flocks found infected with SE or ST, the positive ELISA results appeared later than the bacteriological detection. Therefore, for the

* Corresponding author. Tel.: +33 2 96 01 01 71; fax: +33 2 96 01 62 73.
E-mail address: e.jouy@ploufragan.afssa.fr (E. Jouy).

0167-5877/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.prevetmed.2005.06.003

detection of chicken flocks recently infected with SE or ST, bacteriological examination currently used in France seems to be more appropriate than this ELISA.
© 2005 Elsevier B.V. All rights reserved.

Keywords: *Salmonella*; Hens; Detection; ELISA; Bacteriology

1. Introduction

In France, the bacteriological detection of *Salmonella* Enteritidis (SE) and *Salmonella* Typhimurium (ST), is compulsory under the National Hygienic and Sanitary Control recommended by the Zoonosis order 92/117 (Anonymous, 1992) and national rules (Anonymous, 1998), for breeder and layer flocks of the *Gallus gallus* species. Currently, one environmental swab and one pool of faeces are collected every 2 months in flocks of breeding hens (hatching eggs), and at 24, 40 and 55 weeks of age for laying hens (table eggs). But, as recommended by the European Directive (Anonymous, 1992), surveillance systems based on a rapid and cheap serological screening (Barrow, 1992, 1994; Feld et al., 2000; Gast et al., 2002) may be recognised if they offer equivalent guarantees to bacteriological examinations.

An Enzyme-Linked Immunosorbent Assay (ELISA) was developed by our laboratory for the detection of both serovars concerned by the Zoonosis order: *S*. Enteritidis and Typhimurium (Fawcett et al., 1991; Kles et al., 1993; Proux et al., 2002). The first task was to calculate the sensitivity, specificity and predictive values of this ELISA on hens experimentally inoculated and, secondly, to compare it with the bacteriological examination used as the reference method for field screening.

2. Materials and methods

2.1. ELISA

This ELISA, initially described by Fawcett et al. (1991) and Kles et al. (1993), was based on lipopolysaccharide (LPS) antigens from *S*. Enteritidis and Typhimurium strains (References L2012 and L2262, Sigma Chemical Co., St Louis, USA) obtained by phenol-extraction. These LPS antigens were adsorbed with anti-*Escherichia coli* antibodies (Reference O:111 B4, Bio-Rad, Marnes-La-Coquette, France) in order to reduce non-specific reactions with *E. coli*. IgG antibodies could be detected from sera and yolks (Proux et al., 2001).

Briefly, the samples were diluted 1/300 in peptone buffered solution (PBS). An alkaline phosphatase-conjugated anti-chicken rabbit IgG (Reference A-9171, Sigma Chemical Co., St Louis, USA) was used at a 1/1000 dilution and the *p*-nitrophenyl phosphate substrate (Reference 104–105, Sigma Chemical Co., St Louis, USA) was diluted 1 μL/mL. Coloration was read using a Dynex MRX Revelation spectrophotometer (Dynex technologies, Chantilly, USA) using the 405 and 490 nm filters. A calibrated optical density (COD) was calculated to eliminate the background with a negative control sera

(OD Sample − ODN)/(ODP − ODN) with ODN and ODP meaning OD for the negative and positive controls, respectively. The positive cut-off was established at 0.150 by Kles et al. (1993).

2.2. Bacteriological method

The two reference methods used in France, described by Humbert and Morvan (2001a,b) for organ and environmental samples, respectively, were used.

Briefly, samples were diluted 1/5 to 1/10 in buffered peptone water (BPW) and incubated at 37 °C for 16–20 h for pre-enrichment. Then, samples were enriched on two different selective media: Müller–Kauffmann tetrathionate broth (1/10 rate of inoculation), incubated at 42 °C for 18–24 h and at choice of the diagnostic laboratory: Rappaport–Vassiliadis (1/100 rate of inoculation – incubated at 42 °C for 18–24 h), or Selenite–Cystine (1/10 rate of inoculation – incubated at 37 °C for 18–24 h), or modified semi-solid Rappaport–Vassiliadis medium (MSRV). The latter was surface inoculated with 0.1 mL of the pre-enrichment (divided in three drops) then incubated at 42 °C for an initial period of 18–24 h, followed by an additional period of 18–24 h if no migration appeared after the first incubation period. Each selective enrichment medium must be isolated on one of the four following solid media: Rambach, SMID, Xylose–Lysine–Tergitol 4 or Hektoen agars. Each agar was incubated at 37 °C for 24 h, followed by 24 h more if no characteristics colonies had appeared after the first incubation period. MSRV was isolated only if a characteristic migration was observed. Two characteristic colonies by plate were biochemically identified and serotyped.

2.3. Experimental study

The sensitivity and the dose effect of the ELISA were studied by use of 142 specific pathogen free (SPF) hens (6–19 weeks old) intramuscularly inoculated with 10^6–10^8 colony forming units (CFU) of SE or ST. Three hundred and eight SPF hens (45 weeks old) were oro-nasally inoculated with 10^9 CFU of SE. Sera were collected 3 weeks after inoculation.

The specificity of the ELISA was evaluated by use of sera collected on 933 SPF or conventional hens (8–19 weeks old) submitted to different treatments. One hundred eighty-seven were inoculated with different oil-adjuvanted vaccines (Kles et al., 1993): *Pasteurella multocida* ($n = 19$), *Haemophilus paragallinarum* ($n = 20$), *Mycoplasma gallisepticum* ($n = 34$), *Coronavirus* ($n = 20$), *Paramyxovirus* type 1 (PMV1, $n = 44$), *Birnavirus* ($n = 20$), PMV1/*Coronavirus* ($n = 10$), PMV1/*Coronavirus*/*Birnavirus* ($n = 10$) and PMV1/*Reovirus*/*Coronavirus* ($n = 10$). One hundred twenty nine were inoculated with different *Salmonella* species: *S*. Agona (O: $\underline{1}$, 4, [5], 12) and Berta (O: $\underline{1}$, 9, 12) sharing common somatic antigens with ST (O: $\underline{1}$, 4, [5], 12) and SE (O: $\underline{1}$, 9, 12), respectively; *S*. Virchow and *S*. Infantis (both O: 6, 7, $\underline{14}$) which do not share any common antigens with ST or SE (Popoff, 2001). *S*. Agona ($n = 25$) and *S*. Virchow ($n = 24$) were inoculated intramuscularly while *S*. Berta ($n = 20$) and *S*. Infantis ($n = 60$) were inoculated per os. Whatever serovar used, each chicken received a single dose of 10^8 *Salmonella*. Sera were collected 3 weeks after inoculation.

The sensitivity of our ELISA was calculated as the number of test-positive hens divided by the total number of hens infected with SE or ST. The specificity of our ELISA was calculated as the number of test-negative hens divided by the total number of non-infected hens with SE or ST. The positive predictive value (PPV) of our ELISA was calculated as the number of infected hens that were test-positive to SE or ST divided by the total number of test-positive hens. The negative predictive value (NPV) was calculated as the number of non-infected hens that tested negative divided by the total number of test-negative hens (Toma et al., 2001).

2.4. Field study

Taking into account the geographical dispersion of poultry flocks in France, bacteriological and serological analyses for the field study were performed by 11 local veterinary diagnostic laboratories. The latter were previously assessed by correct results obtained in a ring trial (Humbert et al., 1999; Proux et al., 1999). Moreover, since it was easier to collect eggs than blood samples in farms with laying hens, the ELISA was used on yolks as previously evaluated (Proux et al., 2001). For the flocks of breeding hens, the ELISA was performed on sera. The bacteriological and serological methods described above were used by all of the 11 local veterinary diagnostic laboratories involved in this study.

The main breeding and laying integrated poultry companies were asked to provide voluntary flocks for this study. Forty-three flocks (27 of breeding hens and 16 of laying hens) were sampled monthly during their economic life (from 18–20 weeks to 55–65 weeks of age). One environmental swab and one pool of faecal samples were collected monthly for bacteriological examination. The environmental swab was a sterile piece of cloth pre-dampened in BPW and used to collect the dust on the maximum of materials in the building. The faecal samples consisted of 60 individual recent faeces evenly collected on the ground or on the faeces conveyor belts. For the serological examination, different samples were collected monthly: 20 yolks were sampled from flocks of laying hens and 20 sera were analysed in flocks of breeding hens. This number of samples was calculated in order to detect at least one seropositive bird in a flock with a minimum of 15% infected animals. The risk of not detecting this infection was 5% (Toma et al., 2001).

The serological and bacteriological samples were unsystematic collected (birds were not identified) in each farm. Flocks were considered as serologically positive when at least one sample out of 20 was positive. A flock was classified as bacteriologically positive when *Salmonella* could be isolated in at least one sample.

When bacteriological and serological results were discordant for 2 consecutive months, the following additional samples were collected. For the bacteriology, 12 environmental swabs were collected: four of pooled faeces, two in nests or cages, two on aeration systems, two on feeding devices and two on eggs transport belt. For the serology, 60 sera were collected in the flocks of breeding hens, and 20 sera and 60 yolks were sampled in the flocks of laying hens.

At the end of the economic life in the 43 flocks, when discordant results between serology and bacteriology were still observed, the 12 additional environmental swabs described above were collected and some animals were slaughtered as follows for further examinations. When a negative serological result occurred whereas SE or ST could be

isolated in the farm, 60 hens were killed. Sera were individually tested and presence of *Salmonella* was checked in the liver, spleen, caeca and ovaries. For each kind of organ, five animals were pooled to form one sample. When a seropositive result was detected without any *Salmonella* isolation, 20 hens were slaughtered for individual serological and bacteriological examinations. If more than one seropositive sample out of 20 during monthly sampling was noted, only 10 animals were slaughtered.

3. Results

3.1. Assessment of sensitivity and specificity by use of experimental data

Regarding the sensitivity, 65.0%, 100% and 100% of birds inoculated by intramuscular route with 10^6, 10^7, 10^8 CFU of SE, respectively, were seropositive. Among the hens inoculated with 10^9 CFU of SE by the oral route, 91.2% were seropositive. For ST, 70.0%, 100% and 100% of birds inoculated by intramuscular route with 10^6, 10^7, 10^8 CFU, respectively, were seropositive (Table 1).

The specificity, calculated using the results from the SPF hens without any treatments and those vaccinated with oil-adjuvanted vaccines, was close to 99%. Moreover, the ELISA based on SE and ST antigens detected 96%, 0%, 29% and 20% of the hens infected with *S.* Agona, *S.* Berta, *S.* Virchow and *S.* Infantis, respectively (Table 2).

Using all the data, the global sensitivity, specificity, PPV and NPV were determined to be 91.1%, 95.0%, 89.7% and 95.7%, respectively (Table 3).

3.2. Comparison of bacteriology and serology by use of field data

3.2.1. Bacteriologically negative flocks

Out of the 43 flocks, 18 were classified as free of any *Salmonella* serovar. Among them, 16 (four flocks of laying hens and 12 flocks of breeding hens) were found serologically negative during the whole surveillance period. Two flocks of breeding hens were

Table 1
Sensitivity of ELISA based on *Salmonella* Enteritidis and *Salmonella* Typhimurium antigens according to level and way of inoculation assessed in an experimental study on 450 hens (AFSSA – Ploufragan, France, 1999)

Salmonella	Level (CFU[a]) and inoculation route	No. of hens tested in an ELISA		Sensitivity (%)	Confidence intervals (95%)
		Test-positive	Test-negative		
Enteritidis	10^6 IM[b]	13	7	65.0	[43.7–95.2]
	10^7 IM	48	0	100	–
	10^8 IM	30	0	100	–
	10^9 PO[c]	281	27	91.2	[89.6–92.8]
Typhimurium	10^6 IM	14	6	70.0	[59.8–80.2]
	10^7 IM	8	0	100	–
	10^8 IM	16	0	100	–

[a] Colony forming units.
[b] Intramuscular inoculation.
[c] Per os inoculation.

Table 2
Specificity of ELISA based on *Salmonella* Enteritidis and *Salmonella* Typhimurium antigens according to treatment assessed in an experimental study on 933 hens (AFSSA – Ploufragan, France, 1993–1999)

Treatment	Common antigens with SE or ST	Level (CFU[a]) and inoculation route	Origin of hen	No. of hens tested in an ELISA		Specificity (%)	Confidence intervals (95%)
				Test-positive	Test-negative		
No	–	–	SPF[b]	2	615	99.7	[99.4–99.9]
Oil-adjuved vaccines[c] (Kles et al., 1993)	–	–	SPF	2	185	98.9	[98.1–99.7]
S. Agona	Yes	10^8 IM[d]	SPF	24	1	4.0	[0.08–7.9]
S. Berta	Yes	10^8 PO[e]	Conv.[f]	0	20	100	–
S. Virchow	No	10^8 IM	SPF	7	17	70.8	[61.5–80.1]
S. Infantis	No	10^8 PO	Conv.	12	48	80.0	[74.8–85.2]

[a] Colony forming units.
[b] Specific pathogen free.
[c] Vaccines with oil adjuvant were against: *Pasteurella multocida* (0+/19), *Haemophilus paragallinarum* (1+/20), *Mycoplasma gallisepticum* (0+/34), *Coronavirus* (0+/20), *Paramyxovirus* type 1 – PMV1 (0+/44), *Birnavirus* (0+/20), PMV1 and *Coronavirus* (0+/10), PMV1 *Coronavirus* and *Birnavirus* (1+/10), PMV1 *Reovirus* and *Coronavirus* (0+/10).
[d] Intramuscular inoculation.
[e] Per os inoculation.
[f] Conventional hens.

Table 3
Positive and negative predictive values of ELISA to detect antiobodies against *Salmonella* Enteritidis and *Salmonella* Typhimurium for 450–933 hens (AFSSA – Ploufragan, France, 1993–1999)

ELISA	Number of hens		Total		Confidence intervals (95%)
	Inoculated with SE or ST[a]	Free of SE and ST[b]			
Test-positive	410	47	457	PPV[c]: 89.7%	[88.3–91.1]
Test-negative	40	886	926	NPV[d]: 95.7%	[95.0–96.3]
Total	450	933			
	Sensitivity: 91.1%	Specificity: 95.0%			
Confidence intervals (95%)	[89.8–92.4]	[94.3–95.7]			

[a] Number of hens inoculated by SE ($n = 406$) or ST ($n = 44$).
[b] Number of hens free for all *Salmonella* ($n = 617$), or immunized with oil-adjuved vaccines not against *Salmonella* ($n = 187$) or inoculated with *Salmonella* serovars different from SE or ST ($n = 129$).
[c] Positive predictive value.
[d] Negative predictive value.

serologically tested positive from the beginning of the animal economic life (one–five positive out of 20 samples). However, neither the routine bacteriological samples, nor the additional ones collected because of the discordance between bacteriological and serological results, allowed us to detect any *Salmonella* serovar.

3.2.2. Bacteriologically positive flocks

Out of the 43 flocks, 18 were infected with serovars different from SE and ST (Table 4). These flocks were infected with serovars sharing (groups 1 and 2), or not (groups 3 and 4), common somatic antigens with SE or ST. Four flocks were continually infected with *S.* Heidelberg or *S.* Agona, both serovars belonging to the same serogroup as ST, and were serologically positive (group 2). Among the flocks infected with *Salmonella* serovars which did not share any common somatic antigens with SE or ST, the only three seropositive flocks (group 4) were infected with *S.* Hadar.

S. Enteritidis was found in two flocks of breeding hens and two flocks of laying hens. For the first breeder flock, the bacteriological and serological results were negative until *S.* Enteritidis was found in the hatchery where the eggs of this flock were incubated. After examinations of organs from 60 hens, *S.* Enteritidis was detected in a group of five organs. Consequently, the hens of this flock were prematurely slaughtered in accordance with the French legislation (Anonymous, 1998). For the second breeder flock, SE was isolated only once, on the environmental swab. The other bacteriological and serological samples remained negative until the end of the economic life. The two *Salmonella* Enteritidis infected laying hen flocks were related to human food poisoning. These flocks were belatedly included in our study, at 50 weeks of age. This explains why serological examinations were only performed from that time, whereas bacteriological results were available from 40 weeks of age (Table 5). These two flocks were located on the same farm. The first one was bacteriologically and serologically positive at 50 weeks of age. The second flock was serologically negative but bacteriologically positive at 50 weeks, but positive serological results were then detected at 56 weeks. At the end of production, bacteriological and serological results were concordant for both flocks.

Table 4
Comparison of bacteriological and serological result from 18 flocks of laying and breeding hens contaminated by *Salmonella* serovars different from Enteritidis or Typhimurium (AFSSA – Ploufragan, France, 2001)

Flock	*Salmonella* serovar(s) found in the flock	Common antigens with SE or ST	Found every month in the environment	Serological result	Group
Layer	Saint Paul	Yes	No	Negative	1
Layer	Agona	Yes	No	Negative	
Breeder	Heidelberg	Yes	No	Negative	
Breeder	Coeln	Yes	No	Negative	
Breeder	Heidelberg	Yes	Yes	Positive	2
Breeder	Agona	Yes	Yes	Positive	
Breeder	Heidelberg	Yes	Yes	Positive	
Breeder	Heidelberg	Yes	Yes	Positive	
Layer	Mbandaka	No	No	Negative	3
Layer	O: 43: lv: z53	No	No	Negative	
Layer	Hadar and Infantis	No	No	Negative	
Layer	Yoruba	No	No	Negative	
Layer	Yoruba	No	No	Negative	
Breeder	Montevideo	No	No	Negative	
Breeder	Mbandaka	No	No	Negative	
Breeder	Hadar	No	No	Positive	4
Breeder	Hadar	No	No	Positive	
Breeder	Hadar	No	No	Positive	

S. Typhimurium was detected at the end of the economic life in three flocks of laying hens. For two of them, positive serological results were inconsistently detected (Table 6).

4. Discussion

In experimental conditions, this ELISA showed a sensitivity greater than 90%, calculated by use of sera obtained after inoculation of hens with SE or ST. The level of the serological response was correlated to the inoculation dose.

The specificity of this serological method was 95% in experimental conditions but decreased to 75% in the field study. Indeed, out of the 36 flocks without SE or ST, nine were seropositive. So, this ELISA was not specific for SE or ST, as sera from conventional animals raised in batteries and in contact with various serovars were found positive. Some of these serovars share common antigens with SE or ST, like *S*. Agona and *S*. Heidelberg; but others do not, like *S*. Hadar. Differences in stimulation of the immune system may also be related to the invasive potential of each strain (Desmidt et al., 1998; Roy et al., 2001). These three serovars isolated in our study from breeder flocks were also probably transmitted via the eggshells to the progeny in the hatchery. Indeed, the owners of the breeder flocks infected with *S*. Heidelberg and *S*. Hadar involved in this study (Table 4) reported that the corresponding broiler flocks were frequently infected with the same serovars. Positive serological reactions may also be explained by the possible presence of SE or ST hidden by other serovars in the same sample, as described by Van Winsen et al. (1999) on artificially infected pigs. The serovars Agona, Heidelberg and Hadar are not

Table 5
Comparison of bacteriological and serological result from two layer flocks contaminated by *Salmonella* Enteritidis (AFSSA – Ploufragan, France, 2001)

Hens age (weeks)	Flock 1		Flock 2	
	Bacteriology No. of positive swabs/no. of swabs analysed	Serology No. of positive yolks/no. of yolks analysed	Bacteriology No. of positive swabs/no. of swabs analysed	Serology No. of positive yolks/no. of yolks analysed
Veterinary services sampling				
40	7/7	–	0/7	–
41	–	–	5/14	–
42	–	–	2/28	–
AFSSA sampling				
50	1[a]/2	10/20	1[a]/2	0/20
54	2/2	6/20	2/2	0/20
56[b]	–	–	14/15	3/60 yolks 4/60 sera
59	2/2	9/20	1[a]/2	2/20
65	2/2	7/17	2/2	3/15
End of production				

[a] The positive sample was the pooled faeces.
[b] Additional examinations of a greater number of samples because of discordance between bacteriological and serological results in the two preceding series.

Table 6
Comparison of bacteriological and serological result from three layer flocks contaminated by *Salmonella* Typhimurium (AFSSA – Ploufragan, France, 2001)

Monthly surveillance	Flock 1		Flock 2		Flock 3	
	Bacteriology No. of positive swabs/no. of swabs analysed	Serology No. of positive yolks/no. of yolks analysed	Bacteriology No. of positive swabs/no. of swabs analysed	Serology No. of positive yolks/no. of yolks analysed	Bacteriology No. of positive swabs/no. of swabs analysed	Serology No. of positive yolks/no. of yolks analysed
M1–M6	0/2	0/20	0/2	0/20	0/2	0/20
M7	1[a]/2	0/20	0/2	0/20	0/2	0/20
M8	1[b]/2	0/20	0/2	0/20	2/2	0/20
M9	1[a]/2	2/20	1[a]/2	0/20	2/2	1/20
M10	0/2	0/20	0/2	0/20	End of production	
M11	1[b]/12[c]	0/20	1[a]/2	0/20		
		End of production				

[a] The positive sample was the environmental swab (surfaces and dust).
[b] The positive sample was the pooled faeces.
[c] A great number of swabs was carried out at the end of the production.

currently included in the French legislation. Although they have not the same importance as SE and ST in food poisoning, outbreaks related to these three serovars are reported in different countries (Synnott et al., 1998; Bisbini et al., 2000; Van Look et al., 2000; Lindqvist et al., 2002; Demczuk et al., 2003; Hennessy et al., 2004).

The two seropositive flocks with negative bacteriological results (routine and additional examinations) could be explained by a non-specific response of the ELISA or a possible antimicrobial treatment (Desmidt et al., 1996; Feld et al., 2000).

In flocks infected with SE or ST, the bacteriological examination was found positive before the ELISA. The difference was probably related to the delay in the transmission of *Salmonella* within the flock and the immune response of the birds (Skov et al., 2002). This phenomenon was particularly observed for one of the laying hen flocks infected with SE (Table 5, flock 2). SE was also recovered in two flocks of breeding hens but apparently without massive infection of the birds, since the serological results in these two flocks were always negative. In one of the flocks infected with ST (Table 6, flock 1), there were several consecutive positive bacteriological results but only one positive serological result. In this flock, ST was probably established in the environment without a large infection of the hens at the time of slaughtering. With regards to the second flock infected with ST, the absence of positive serological results could be explained by the slaughtering of the hens before the infection of birds occurred. In the third flock, the serology became positive 1 month after the first detection of ST by bacteriological method. This result could not be confirmed because the flock reached the end of its economic life and the hens had to be slaughtered.

On a national basis, the use of this ELISA for the detection of flocks recently infected with *Salmonella* could not be recommended. Indeed, it may induce a delayed elimination of positive flocks and so may contribute to food poisoning and environmental dissemination of *Salmonella*.

Nevertheless, serology remains interesting to survey a large scale of poultry flocks because of its possible automation, low cost and ability to detect chronic infections without *Salmonella* excretion (Cooper et al., 1989; Hassan et al., 1990; Nicholas and Cullen, 1991; Barrow, 1992, 1994; Desmidt et al., 1996; Skov et al., 2002). If a country decides to use a serological method in its surveillance system, a positive response in a flock without any vaccination against *Salmonella* will need further bacteriological examination. This confirmation remains necessary because of a possible non-specific response of the ELISA which may lead to an unjustified chicken slaughtering and an economic loss for the farmer. The bacteriological confirmation is also necessary for epidemiological reasons (Skov et al., 2002) and regulations: Enteritidis and Typhimurium are currently the only two serovars targeted by the French authorities. Other serovars could be targeted since the European Regulation 2160/2003 (Anonymous, 2003). Consequently, the bacteriological examination is still more suitable than our ELISA to follow the evolution of the legislation.

For a recent infection of chicken flocks with SE and ST, the bacteriological method currently used in France seem to be an earlier and a more reliable indicator than the ELISA used in this study. Further investigations are necessary with chronic infected flocks and hens vaccinated against *Salmonella*. So, for the time being, the French national surveillance of *S*. Enteritidis and *S*. Typhimurium in breeder and layer flocks of *Gallus gallus* species should remain entirely based on bacteriological examinations.

Acknowledgements

This study was supported by a grant from the European Union and the Brittany region (FEOGA). The authors thank all the people from UGPVB (Union of meat-producing companies), diagnostic laboratories, hatcheries owners, farmers and veterinarians involved in this work. We gratefully acknowledge Dr. Cyril Barbezange for his helpful comments.

References

Anonymous, 1992. European Directive 92/117/CEE concerning the protection measures against some zoonoses and zoonotic agents in the animals and in the products of animal origin, in order to prevent the infection areas and food poisoning due to foodstuffs. Offic. J. Eur. Commun. L62, 38–48.

Anonymous, 1998. Decrees of October 26th concerning the measures against *Salmonella* Enteritidis and *Salmonella* Typhimurium infections in *Gallus gallus* specie for meat breeders and production of consumption eggs. Offic. J. French Repub. 284, 18444–18453.

Anonymous, 2003. Regulation No 2160/2003 of the European Parliament and of the council of 17 November 2003 on the control of *Salmonella* and other specified food-borne zoonotic agents. Offic. J. Eur. Union L325, 1–15.

Barrow, P.A., 1992. ELISAs and the serological analysis of *Salmonella* infections in poultry: a review. Epidemiol. Infect. 109, 361–369.

Barrow, P.A., 1994. Serological diagnosis of *Salmonella* serotype Enteritidis infections in poultry by ELISA and other tests. Int. J. Food Microbiol. 21, 55–68.

Bisbini, P., Leoni, E., Nanetti, A., 2000. An outbreak of *Salmonella* Hadar associated with roast rabbit in a restaurant. Eur. J. Epidemiol. 16, 613–618.

Cooper, G.L., Nicholas, R.A., Bracewell, C.D., 1989. Serological and bacteriological investigations of chickens from flocks naturally infected with *Salmonella* Enteritidis. Vet. Rec. 125, 567–572.

Demczuk, W., Soule, G., Clark, C., Ackermann, H.W., Easy, R., Khakhria, R., Rodgers, F., Ahmed, R., 2003. Phage-based typing scheme for *Salmonella* enterica serovar Heidelberg, a causative agent of food poisonings in Canada. J. Clin. Microbiol. 41, 4279–4284.

Desmidt, M., Ducatelle, R., Haesebrouck, F., De Groot, P.A., Verlinden, M., Wijffels, R., Hinton, M., Bale, J.A., Allen, V.M., 1996. Detection of antibodies to *Salmonella* Enteritidis in sera and yolks from experimentally and naturally infected chickens. Vet. Rec. 138, 223–226.

Desmidt, M., Ducatelle, R., Haesebrouck, F., 1998. Serological and bacteriological observations on experimental infection with *Salmonella* Hadar in chickens. Vet. Microbiol. 60, 259–269.

Fawcett, P.T., Gibney, K.M., Rose, C.D., Klein, J.D., Doughty, R.A., 1991. Adsorption with a soluble *E. coli* antigen fraction improves the specificity of ELISA tests for Lyme Disease. J. Rheumatol. 18, 705–708.

Feld, N.C., Ekeroth, L., Gradel, K.O., Kabell, S., Madsen, M., 2000. Evaluation of a serological *Salmonella* mix-ELISA for poultry used in a national surveillance programme. Epidemiol. Infect. 125, 263–268.

Gast, R.K., Nasir, M.S., Jolley, M.E., Holt, P.S., Stone, H.D., 2002. Serologic detection of experimental *Salmonella* Enteritidis infections in laying hens by fluorescence polarization and enzyme immunoassay. Avian Dis. 46, 137–142.

Hassan, J.O., Barrow, P.A., Mockett, A.P., Mcleod, S., 1990. Antibody response to experimental *Salmonella* Typhimurium infection in chickens measured by ELISA. Vet. Rec. 126, 519–522.

Hennessy, T.W., Cheng, L.H., Kassenborg, H., Ahuja, S.D., Mohle-Boetani, J., Marcus, R., Shiferaw, B., Angulo, F.J., Emerging Infections Program FoodNet Working Group, 2004. Egg consumption is the principal risk factor for sporadic *Salmonella* serotype Heidelberg infections: a case-control study in FoodNet sites. Clin. Infect. Dis. 38 (Suppl. 3), S237–S243.

Humbert, F., Jouy, E., Proux, K., 1999. Bacteriologic collaborative study with 11 laboratories in France. In: Fourth workshop CRL-*Salmonella*, RIVM, Bilthoven, The Netherlands, November 4–5.

Humbert, F., Morvan, H., 2001a. Isolation and identification of *Salmonella* in poultry organs. French Standard BA 60 for Laboratory Certification (COFRAC/116/BA60).

Humbert, F., Morvan, H., 2001b. Isolation and identification of *Salmonella* in environmental samples. French Standard BA 70 for Laboratory Certification (COFRAC/116/BA70).

Kles, V., Morin, M., Humbert, F., Lalande, F., Guittet, M., Bennejean, G., 1993. Serologic diagnosis of avian salmonelloses: adjustment of an ELISA test using antigens adsorbed with the aid of anti-colibacillary sera. Zentralbl Veterinarmed B 40, 305–325.

Lindqvist, N., Siitonen, A., Pelkonen, S., 2002. Molecular follow-up of *Salmonella* enterica subsp. enterica serovar Agona infection in cattle and humans. J. Clin. Microbiol. 40, 3648–3653.

Nicholas, R.A., Cullen, G.A., 1991. Development and application of an ELISA for detecting antibodies to *Salmonella* Enteritidis in chicken flocks. Vet. Rec. 128, 74–76.

Popoff, M.Y., 2001. Antigenic formulas of the *Salmonella* serovars. In: WHO Collaborating Centre for Reference and Research on *Salmonella*, eighth ed. Institut Pasteur, Paris, France.

Proux, K., Jouy, E., Humbert, F., 1999. Immunologic collaborative study with 11 laboratories in France. In: Fourth workshop CRL-*Salmonella*, RIVM, Bilthoven, The Netherlands, November 4–5.

Proux, K., Jouy, E., Houdayer, C., Oger, A., Protais, J., Dibb-Fuller, M., Boscher, E., Gillard, A., Beaumont, C., Duchet-Suchaux, M., 2001. Comparison of anti-*Salmonella* Enteritidis antibodies in hen sera and egg yolks. In: Proceedings of the 2001 Spring Meetings of the WPSA French and WPSA United Kingdom Branches. Br. Poult. Sci. 42 (Suppl.), 35–36.

Proux, K., Jouy, E., Houdayer, C., Protais, J., Dibb-Fuller, M., Boscher, E., Gillard, A., Gracieux, P., Gilbert, F., Beaumont, C., Duchet-Suchaux, M., 2002. Reliable ELISAs showing differences between resistant and susceptible lines in hens orally inoculated with *Salmonella* Enteritidis. Vet. Res. 33, 23–33.

Roy, P., Dhillon, A.S., Shivaprasad, H.L., Schaberg, D.M., Bandli, D., Johnson, S., 2001. Pathogenicity of different serogroups of avian *Salmonellae* in specific-pathogen-free chickens. Avian Dis. 45, 922–937.

Skov, M.N., Feld, N.C., Carstensen, B., Madsen, M., 2002. The serologic response to *Salmonella* Enteritidis and *Salmonella* Typhimurium in experimentally infected chickens, followed by an indirect lipopolysaccharide enzyme-linked immunosorbent assay and bacteriologic examinations through a one-year period. Avian Dis. 46, 265–273.

Synnott, M.B., Brindley, M., Gray, J., Dawson, J.K., 1998. An outbreak of *Salmonella* Agona infection associated with precooked turkey meat. Commun. Dis. Public Health 1, 176–179.

Toma, B., Dufour, B., Sanaa, M., Benet, J.J., Shaw, A., Moutou, F., Louza, A., 2001. Epidémiologie appliquée à la lutte collective contre les maladies animales transmissibles majeures. AEEMA, deuxième éd., pp. 52–68, 141–158.

Van Look, F., Ducoffre, G., Dumont, J.M., Libotte-Chasseur, M.L., Imberechts, H., Gouffaux, M., Houins-Roulet, J., Lamsens, G., De Schrijver, K., Bin, N., Moreau, A., De Zutter, L., Daube, G., 2000. Analysis of foodborne disease in Belgium in 1997. Acta Clin. Belg. 55, 300–306.

Van Winsen, R.L., Swanenburg, M., Snijders, J.M.A., Urlings, H.A.P., 1999. Recovery of *Salmonella* serotypes from swine faecal samples using ISO 6579. In: Proceedings of the third international symposium on the epidemiology and control of *Salmonella* in pork. Washington DC, USA, pp. 90–91.