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Research Note

Three-Year Surveillance Program Examining the Prevalence of *Campylobacter* and *Salmonella* in Whole Retail Raw Chicken

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ABSTRACT

A 36-month study of *Campylobacter* and *Salmonella* in retail raw whole chicken was carried out to measure baseline rates at the retail level, establish seasonality, and observe changes in rates over time. In total, 2,228 samples were taken between November 2001 and December 2004. The *Campylobacter* rate was unchanged over the 3 years of the study, but the *Salmonella* rates declined significantly between 2001 and 2004. There was also some seasonality in *Campylobacter* rates in fresh samples. The overall conclusion from the study was that the *Salmonella* rate in raw chicken available to consumers in Wales fell significantly between 2001 and 2004, while the *Campylobacter* rate remained unchanged and is still by far the greater problem.

In the United Kingdom, *Campylobacter* is the most common bacterial gastrointestinal pathogen, with 49,050 reports of human infection recorded during 2003 *(5).* It is recognized that the reporting level of human infections at the laboratory level is likely to be a small proportion of the actual cases, and it has been estimated that the number of actual cases in the United Kingdom during 2003 was approximately 400,000 *(5).* Human *Campylobacter* infection rates in temperate countries have a well-characterized and consistent seasonality. In Wales, there were human infection peaks in May 2002 (week 20), May 2003 (week 23), and June 2004 (week 24) *(4).* Peaks in human infections have also been reported in Scandinavian countries and New Zealand *(12).* In European countries, the seasonal peaks varied between weeks 22 and 33, with more northerly countries having later peaks *(12).*

There were 16,343 cases of salmonellosis reported in the United Kingdom during 2003, a significant decrease in cases since the peak during the 1990s, but a slight increase compared with figures reported in 2001 and 2002 *(5).* Eggs and products containing eggs accounted for 10 United Kingdom outbreaks during 2003, and poultry accounted for three *(5).* United Kingdom surveys indicated a contamination rate of 0.3% for *Salmonella (6)* in shell eggs, whereas for raw poultry meat the rate was 5.7% *(5).* In Wales, the annual rates of confirmed laboratory reports have declined since the mid-1990s, but in 2003 the rate increased compared with 2002 *(3)* in parallel with the rest of the United Kingdom.

Raw chicken and poultry meat is still recognized in the United Kingdom and most other developed countries as a significant source of both pathogens *(9, 14),* and consumption and handling of chicken is well recognized as a risk factor for human *Campylobacter* infection *(7).* A recent study in Northern Ireland found that 67.7% of 387 flocks tested were positive for *Campylobacter (13).* Recent retail-level studies have found contamination rates for *Campylobacter* of 68 and 73.1% in Wales *(9, 11),* 83.3% in England *(10),* and 57% in Northern Ireland *(15).*

The objectives of this work were to establish baseline levels of *Campylobacter* and *Salmonella* in randomly sampled raw whole retail chicken available to consumers in Wales, to assess if there were any significant changes in overall positive rates between years, and to observe any seasonality in positive rates.

MATERIALS AND METHODS

Sampling and selection of premises. Fresh or frozen raw whole chickens were purchased on a weekly basis from retailers selected at random by environmental health officers from 22 local authorities in Wales. Sampling took place between November 2001 and December 2004. The sampling target was to obtain 70% of samples from supermarkets and retail grocery stores and 30% from local independent butchers, of which 75% were fresh and 25% were frozen. These targets were in line with a previous national survey carried out by the Food Standards Agency and reflected the retail market share. All samples were purchased directly from chilled and frozen cabinets or displays and transported to the laboratory in insulated cool boxes with ice packs. Information was gathered on producers either from the labels or from questioning the proprietors.

Sample preparation. All laboratories used methods accredited by the United Kingdom Accreditation Service. Chickens were

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stored in sterile metal trays at <5°C before examination, and frozen samples were allowed to defrost. All fresh samples were examined within 24 h of receipt. For examination, the neck skin was removed and divided into two equal portions with a sterile scalpel. The carcass was then placed into a sterile bag and manually rinsed for 2 min in 225 ml of buffered peptone water, ensuring that all surfaces, internal and external, had contact with the rinse. The rinse was then poured into a sterile jar and a portion of neck skin added. Twenty-five milliliters (10%) of this rinse was then pipetted into 225 ml of *Campylobacter* enrichment broth (CEB) leaving 200 ml (90%) buffered peptone water. The remaining part of the neck skin was then added to the CEB. All media were supplied by Oxoid, Basingstoke, UK.

***Campylobacter* examination method.** The method used was issued in a Standard Operating Procedure by the Health Protection Agency, which itself was based upon the method described in BS 5763: Part 17: 1996, ISO: 1995 *(1)*. The CEB was incubated under microaerophilic conditions for 24 h (±6 h) at 37°C (±1°C), followed by incubation at 41.5°C (±1°C) for 24 h (±6 h). The CEB was subcultured onto charcoal cefoperazone desoxycholate agar plates and incubated in a microaerophilic atmosphere (Campygen, Oxoid) at 37°C (±1°C) for 48 h (±6 h). All media were supplied by Oxoid. Presumptive positive colonies were confirmed by oxidase reaction, growth under microaerophilic conditions, lack of growth under aerobic conditions, microscopic determination of cell morphology using carbol fuchsin–stained preparations, and examination for typical cells with the oil immersion lens (×400 magnification).

***Salmonella* examination method.** The method used was issued in a Standard Operating Procedure by the Health Protection Agency, which itself was based upon the method described in BS EN 12824: 1998 *(2)*. The buffered peptone water was incubated for 18 to 24 h at 37°C (±1°C), followed by selective enrichment of 0.1 ml in 10 ml of Rappaport-Vassiliadis and of 1 ml in 9 ml of selenite cystine broth. The Rappaport-Vassiliadis broth was incubated at 42°C (±1°C) for 18 to 24 h, and the selenite cystine broth was incubated at 37°C (±1°C) for 18 to 24 h. The broths were then subcultured onto brilliant green agar and xylose lysine desoxycholate agar and incubated at 37°C (±1°C) for 18 to 24 h. All media were supplied by Oxoid. Presumptive positive colonies (nonlactose fermenting with suitable colony morphology) were then confirmed by serological (polyvalent O and polyvalent H antigens, Murex Biotech, Dartford, UK, and ProLab, Neston, UK) and biochemical tests (API 20E, bioMérieux, Marcy l'Etoile, France).

Statistical analysis. Data were analyzed with the statistical calculator function of an adapted version of the Epi Info program. Hypothesis testing was carried using the χ^2 test. The 5% significance level was used as the alpha level. The null hypothesis was that there was no difference between the positive percentage rates for *Salmonella* and *Campylobacter* for overall rates, for fresh and frozen samples, or for samples taken from retailers and butchers between years.

RESULTS

In total, 2,228 raw chickens were sampled between November 2001 and December 2004. When the rates between the sampling periods were considered (Table 1), the *Salmonella* rate showed a significant decline between 2001 and 2004, with the rate falling from 8.4 to 4.9%. This significance is reflected when fresh samples and samples from

TABLE 1. *Overview of baseline percent positive rates by year sampled for* Campylobacter *and* Salmonella *from all samples, for samples taken from retailers or butchers, and for fresh and frozen samples and overview of significance (P-values) for differences in percent positive rates between sampling periods for each parameter*

Year	Overall positive rate (%)		Positive rate from retailers (%)		Positive rate from butchers (%)		Positive rate for fresh (%)		Positive rate for frozen (%)	
	Campylobacter	*Salmonella*	*Campylobacter*	*Salmonella*	*Campylobacter*	*Salmonella*	*Campylobacter*	*Salmonella*	*Campylobacter*	*Salmonella*
2001 and 2002	523/739 (70.8)	62/739 (8.4)	368/519 (70.9)	47/519 (9.1)	155/220 (70.4)	15/220 (6.8)	388/553 (70.2)	44/553 (8.0)	135/186 (72.6)	18/186 (9.7)
2003	538/736 (73.1)	42/736 (5.7)	416/565 (73.6)	39/565 (6.9)	120/171 (70.1)	3/171 (1.8)	400/544 (73.5)	24/544 (4.4)	138/192 (71.9)	18/192 (9.4)
2004	517/753 (68.6)	37/753 (4.9)	399/583 (68.4)	31/583 (5.3)	118/170 (69.4)	6/170 (3.5)	415/578 (71.8)	27/578 (4.7)	101/173 (58.4)	10/173 (5.8)
P-value[a]	0.228	0.016	0.153	0.052	0.975	0.042	0.464	0.018	0.005	0.335

[a] *P*-values refer to comparison of data between years for all samples, samples from retailers, samples from butchers, and fresh and frozen samples.

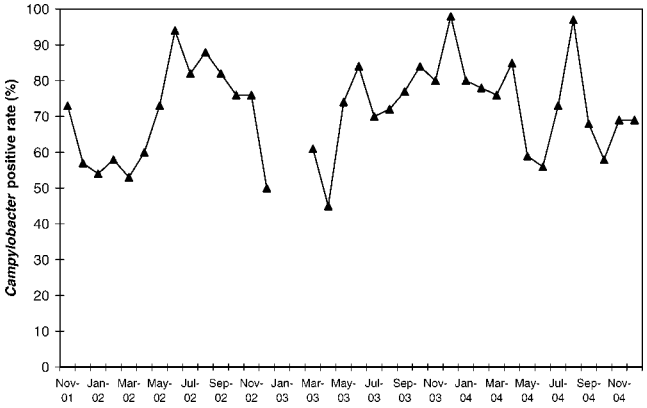

FIGURE 1. *Overview of seasonality of* Campylobacter *positive rates in fresh samples sampled between 2001 and 2004.*

butchers were considered, both of which also showed a significant decline in rates between 2001 and 2004. Frozen samples, although showing a decline in rates from 9.7 to 5.8%, did not have statistical significance ($P = 0.335$). Samples taken from retailers also showed a decline in rate from 9.1 to 5.3%, but the *P*-value of 0.052 was inconclusive in terms of significance. For *Campylobacter*, none of the rates altered significantly between 2001 and 2004, except for frozen samples, where there was a significant decline in rate between 2003 and 2004 (73 to 58%).

In terms of seasonality of the *Campylobacter* isolation rate for fresh samples taken in Wales, there was distinct seasonality noted (Fig. 1). For 2002, the peak rate occurred in June, whereas in 2003 there were two peaks (June and December) and in 2004 there was one main peak in August and a smaller one in April.

DISCUSSION

This study was composed of three separate surveys: a 14-month survey in 2001 and 2002, a 10-month survey in 2003, and a 12-month survey in 2004. Essentially, this work spanned the period from the end of 2001 to the end of 2004, with only a 2-month break in sampling in January and February 2003, which occurred for logistical reasons. This surveillance has benefited from the use of common sampling and examination methods that have remained unchanged over the period of the study. When the sources of the chickens were examined, it was found that the vast majority of chickens sampled had United Kingdom producer codes (results not shown). There were some large producers whose products were sampled hundreds of times, but there were also some small local producers who were only sampled in very small numbers. This was recognized as an expected part of the survey and was considered to reflect the choices that consumers had.

When the rates from the three sampling periods were compared, it was found that, for the overall *Campylobacter* contamination rate, there had been no significant annual decrease in the percentage of positive levels. In contrast, *Salmonella* rates had declined since 2001, with a decrease of approximately 42%

The rates found for *Campylobacter* are consistent with other published retail surveys. *Campylobacter* rates of 57, 68, 73, and 83% have all been reported in the United Kingdom (*9–11, 14*). The United Kingdom Food Standards Agency has recently reviewed the evidence and has set a baseline for *Campylobacter* rate in chicken at 70%, a rate that is consistent with the findings from this surveillance *(8)*. For the *Salmonella* rates found, other published United Kingdom surveys have reported rates of 29 and 11% *(9, 15)*, which are higher than the rate found in this survey and may reflect the improvements in the *Salmonella* contamination of raw poultry made in recent years.

For the comparison of the monthly *Campylobacter* rates, it should be noted that frozen samples were excluded from this comparison. The reason was that, although the contamination rate of fresh samples can be directly related to a specific time period, the same cannot be done for frozen samples because there is no easily definable temporal relationship between slaughter date and sampling date. For fresh chickens, the period between slaughter and expiry of use-by date was assumed to have been 7 to 10 days, and therefore the contamination status of a fresh bird could be directly related to a specific week or month and all the birds sampled in that period could be compared. There were some distinct patterns, with peak rates in June 2002, June 2003, December 2003, and August 2004. These peaks may provide clues to the source of the contamination of birds. The data on the occurrence of peaks also make a strong case for suggesting that short-term snapshot surveys of *Campylobacter* in fresh chicken should not be carried out in case the time period chosen for sampling skewed the rates found.

In conclusion, this 3-year program of surveying the contamination rates in raw whole retail chicken available to consumers has helped to identify that the positive rates of *Salmonella* contamination are showing significant improvement but that *Campylobacter* rates are not declining and are still approximately 70%.

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