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Spatial and temporal patterns in antimicrobial resistance of *Salmonella* Typhimurium in cattle in England and Wales

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SUMMARY

Salmonella is the second most commonly reported human foodborne pathogen in England and Wales, and antimicrobial-resistant strains of *Salmonella* are an increasing problem in both human and veterinary medicine. In this work we used a generalized linear spatial model to estimate the spatial and temporal patterns of antimicrobial resistance in *Salmonella* Typhimurium in England and Wales. Of the antimicrobials considered we found a common peak in the probability that an *S*. Typhimurium incident will show resistance to a given antimicrobial in late spring and in mid to late autumn; however, for one of the antimicrobials (streptomycin) there was a sharp drop, over the last 18 months of the period of investigation, in the probability of resistance. We also found a higher probability of resistance in North Wales which is consistent across the antimicrobials considered. This information contributes to our understanding of the epidemiology of antimicrobial resistance in *Salmonella*.

Key words: Antimicrobial resistance in agricultural settings, *Salmonella* Typhimurium, spatial modelling.

INTRODUCTION

Salmonella is the second most commonly reported human foodborne pathogen in England and Wales, with more than 10 000 laboratory-confirmed cases in 2009 [1]. The majority of these cases involve foodborne transmission, mostly of animal origin [2]. Antimicrobial-resistant (AMR) serotypes of *Salmonella* are globally widespread [3] and are an increasing problem in human and veterinary medicine [4]; moreover, multidrug resistance (MDR; i.e. resistance to $\geqslant 4$ antimicrobials) has commonly been recorded [3, 5]. Despite legislation to control antimicrobial use, prevalence of AMR *Salmonella* isolates has increased in developed and developing countries in recent years [3, 6]. AMR serotypes can be detrimental to animal health and productivity [5, 7] and the resulting disease can be severe. Clinical salmonellosis in cattle, for example, can cause acute diarrhoea, abortion, decreased milk production and high mortality [8]. It results in economic loss to herd owners and impacts on future trading opportunities [7]. AMR serotypes in livestock can cause human infections through the food chain [3, 9] and therefore also have an adverse impact on human health and welfare [10, 11], e.g. through failed

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treatment or prolonged illness [9], increased hospitalization [12], or increased mortality [13].

The majority of salmonellosis incidents are caused by relatively few serotypes. Serotypes *S*. Typhimurium and *S*. Enteritidis account for 60–80% of all human salmonellosis [1]. *S*. Typhimurium is a particularly epidemic serotype with a ubiquitous host range that is commonly responsible for clinical disease in both livestock and humans [5]. In cattle, for example, it accounts for more than 7% of incidents and is the second most common serotype after *S*. Dublin. (Although *S*. Dublin causes more than 70% of outbreaks it is rarely associated with human foodborne infection [5].) Antimicrobial resistance is a major problem in *S*. Typhimurium compared to other *Salmonella* serotypes [14]. In England and Wales at least 70% of isolates from livestock are resistant to one or more antimicrobials [5]; in humans the proportion is greater than 80% [4]. Multidrug-resistant *S*. Typhimurium was first identified in the UK in the 1960s and a number of different phage types have caused serious epidemics since then [3], e.g. multiresistant definitive phage type (DT) 104 [15].

Previous research has highlighted that there is limited understanding about certain aspects of the development of AMR serotypes, including risk factors for carriage of resistant organisms [16], seasonal prevalence [17] and the emergence of multidrug-resistant serotypes [11, 18]. This lack of knowledge hinders attempts to develop effective targeted *Salmonella* control programmes in the UK and worldwide. Critical to this is a full understanding of the spatial and temporal development of AMR serotypes in livestock populations. Similar antimicrobial resistance patterns occurring at similar periods in time ('temporal components') or locations ('spatial components') may reflect the effect of explanatory variables which themselves are structured in time and/or space [19] or might provide evidence of a contagious mechanism. Large-scale regional variations in infections may indicate large-scale risk factors, e.g. use of certain antimicrobials in certain regions or at certain time of the year, whereas small-scale patterns, e.g. clustering of farms with resistance to the same antimicrobial may indicate a local risk factor, e.g. environmental contamination [20]. While some explanatory variables may be recorded, others may currently be unmeasured or unknown. However, quantitative description of such spatial and temporal patterns will inform knowledge of the underlying epidemiology and biological processes [21].

As a step towards the goal of better understanding the development of antimicrobial resistance, we investigated temporal and spatial patterns of antimicrobial resistance in *S*. Typhimurium isolates from one livestock sector: cattle in England and Wales. We focused on three particular antimicrobials: streptomycin, sulphonamide compounds, and chloramphenicol, as well as examining MDR. Specifically, we address the following questions:

- Is there evidence of changes from year to year in the probability of antimicrobial resistance being observed in a given incident?
- Is there evidence of a seasonal effect in the occurrence of antimicrobial resistance?
- Is there evidence of geographical variation across England and Wales in the probability of resistant organisms being observed in a given incident?
- What are the similarities and differences in the temporal and spatial resistance patterns for the different antimicrobials?

MATERIALS AND METHODS

Data were obtained from the Veterinary Laboratories Agency (VLA) 'Farmfile' database [1], which documents all livestock incidents of *Salmonella* in the UK. The database is one of the UK's most extensive livestock databases (for review see [22]) and includes details of each incident's location, date, *Salmonella* serotype and antimicrobial sensitivity. It is a passive surveillance system; samples are submitted to the VLA regional laboratories by veterinary practitioners. *Salmonella* is a notifiable disease in the UK and therefore all cases are reported. The database does include reports from statutory monitoring and surveillance; however, the majority of these are from poultry flocks where surveillance is common practice in contrast to the majority of reports from other species which are the result of examinations of clinical disease.

Data from 1 January 2003 to 31 December 2006 were used in this analysis. Data were restricted to this time period to strike a compromise between being able to look for seasonality and compatibility of data over time: in January 2003 the VLA established the current version of the 'Farmfile' database which integrated *Salmonella* recording and reporting [5]. Data were selected according to reason for submission to the VLA. We included any incident that was reported as a result of examinations performed to diagnose

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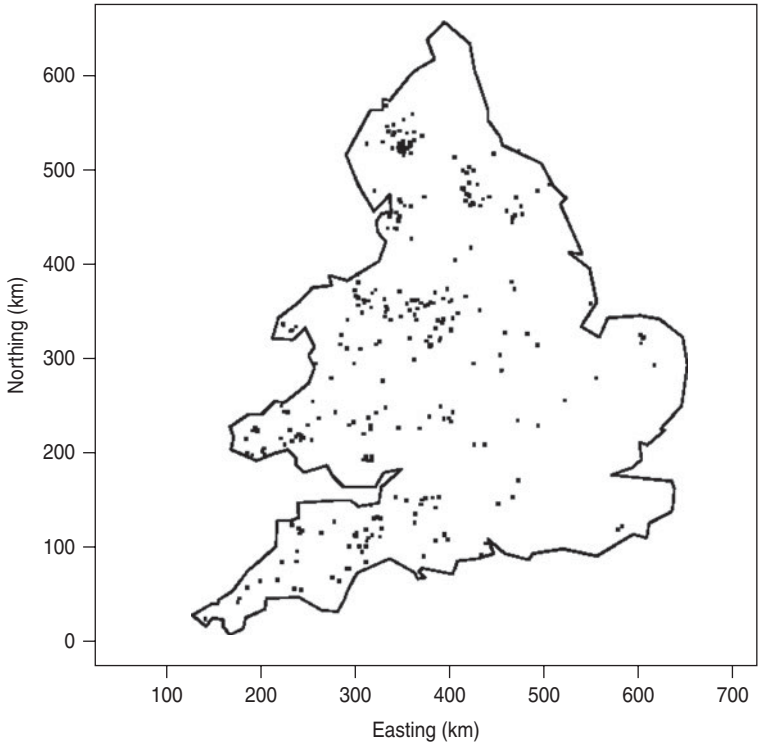

Fig. 1. Locations of the 256 farms which experienced at least one incident of *S*. Typhimurium between January 2003 and December 2006.

clinical disease and excluded any reports that resulted from statutory monitoring or surveillance activities. We focused our analysis on cattle, a species in which isolations must by law be reported and a known reservoir for antimicrobial resistance to *S*. Typhimurium [5]. An incident of *Salmonella* was defined as the first isolation and any subsequent isolations of the same serotype of a particular *Salmonella*, following diagnosis of a clinical case, from an animal, group of animals or their environment on a single premises, within 30 days [5].

Samples from each incident had been tested for their *in vitro* sensitivity to 16 antimicrobials using the British Society for Antimicrobial Chemotherapy (BSAC) disc diffusion technique (www.bsac.org.uk) on Oxoid 'Isosensitest' agar. The method is described in detail in a Defra publication [5]. The antimicrobials (with disc concentrations in parentheses) were ampicillin (10 μg), amoxicillin (30 μg), streptomycin (25 μg), sulphonamide compounds (comprising of 37% sulphadiazine; 37% sulphathiazole and 26% sulphamerazine) (300 μg), chloramphenicol (10 μg), sulphamethoxozole (25 μg), nalidixic acid (30 μg), tetracycline (10 μg), neomycin (10 μg), furazolidone (15 μg), ceftazidime (30 μg), amikacin (30 μg), gentamicin (10 μg), cefotaxime (30 μg), apramycin (15 μg) and ciprofloxacin (1 μg). Separate tests for extended spectrum β-lactamases were not routinely performed until after 2006 and so these data are not included. The choice of antimicrobials, which is reviewed periodically, is designed to comprise a core set which has been used in veterinary practice for many years, some of the more recently licensed antimicrobials and some of limited usage in animals in Great Britain which are used in other European countries. Analysis excluded any incidents where the samples were not tested because there was no approved sensitivity test. We refer to an incident of *S*. Typhimurium as a resistant case if the sample from this incident shows resistance to a certain antimicrobial; otherwise it is a susceptible case. We refer to an incident as multidrug resistant if a sample was resistant to $\geqslant 4$ antimicrobial agents in the panel of 16 [5].

Exploratory data analysis

Between January 2003 and December 2006 there were 294 incidents of *S*. Typhimurium in England and Wales on 256 farms, the locations of which are shown in Figure 1. Of these farms 226 experienced a single incident, 26 farms had two incidents, one farm had three incidents, two farms had four incidents, and one

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Table 1. *Number of* Salmonella *Typhimurium incidents per year and frequency of antimicrobial resistance to each antimicrobial* (*only antimicrobials for which there was at least one incident of resistance are listed*)

Year	2003	2004	2005	2006	Total
S. Typhimurium incidents	80	69	45	100	294
Antimicrobials					
Streptomycin	53	52	28	49	182
Sulphonamide compounds	60	55	31	80	226
Nalidixic acid	11	0	3	0	14
Ampicillin	60	51	33	77	221
Chloramphenicol	57	47	28	75	207
Tetracycline	66	58	33	79	236
Sulphamethoxozole	23	22	6	22	73
Multidrug resistant*	59	51	30	78	218

* Resistant to $\geqslant 4$ of the 16 antimicrobials tested.

farm had five incidents. The largest distance from a farm to its nearest neighbour was 67·9 km. For data protection purposes in all spatial plots the coordinates of each individual farm incident have been randomized uniformly to a disk of radius 10 km centred on the true location.

Table 1 presents the frequencies of *S*. Typhimurium incidents which were resistant or susceptible to each antimicrobial. None of the incidents showed evidence of resistance to amoxicillin, ceftazidime, amikacin, gentamicin, apramycin, or ciprofloxacin; resistance to cefotaxime, furazolidone, and neomycin was found in only one, one, and four of the incidents, respectively, over the 4-year period. These antimicrobials are therefore not included.

We were interested in discerning patterns in the spatial or temporal variability of resistance and in comparing the patterns for antimicrobials between which a genetic link is suspected as well as in comparing compounds where no such relationship is believed to exist. For this reason our analysis focused on streptomycin, sulphonamide compounds, and chloramphenicol, to which 62%, 77%, and 70% of the cattle isolates demonstrated resistance, respectively.

These antimicrobials often occur in the characteristic multiresistant pentavalent-resistant pattern (resistance to tetracycline, ampicillin, chloramphenicol, streptomycin and sulphonamide compounds) due to chromosomal integration [23]. We could not analyse antimicrobials to which isolates were either almost entirely susceptible or almost entirely resistant. Therefore we could not include antimicrobials which are not part of the multiresistant pentavalent pattern, and which are unlinked in their mode of resistance, e.g. nalidixic acid. Independent spatiotemporal analyses was performed on the relative incidences of resistant and susceptible cases for each of the three antimicrobial datasets identified above and for MDR.

Figure 2 maps all of the incidents of *S*. Typhimurium indicating the presence or absence of bacteria susceptible to chloramphenicol. Superficially, at least, plots for streptomycin, sulphonamide compounds, and MDR appear very similar, with, e.g. some separation between groups of resistant samples and groups of non-resistant samples, especially in Wales and the Southwest. Exploratory fitting of Generalized Additive Models (e.g. [24]), showed a likely spatial pattern, motivating the need to allow for spatial correlation in the full statistical analysis.

Statistical framework

We focused on the probability that an observed outbreak of *S*. Typhimurium at time t and farm i is resistant to 'A', where 'A' denotes one of the antimicrobials of interest, or MDR. This quantity, which we denote $p_i^A(t)$ is our main focus since we are interested in the pattern of resistance in observed incidents of *S*. Typhimurium. We were particularly interested in how the chance that a given incident is resistant to 'A' varies across England and Wales as well as over the 4-year period, and so we express this probability as a function of the spatial location of farm i, x_i; i.e. $p^A(x_i,t)=p_t^A(t)$. We studied each antimicrobial (and MDR) in turn, and drop the superscript to ease notation.

A Generalized Linear Spatial Model (GLSM; e.g. [25]) with a logit (i.e. log-odds) link for $p(x_i, t)$ is as follows,

$$\text{logit}(p(x,t))=\beta' f(t)+S(x).$$

Temporal trends are included through the deterministic covariate term $f(t)$ as in a traditional Generalized Linear Model (GLM); however, the GLSM also allows for spatial variation via a spatially structured random-effects term $S(x)$. The time-varying contribution to the log-odds that an incident is resistant to 'A' is assumed to be the same for all farms. Conversely, we assume that there is a spatially varying contribution to the log-odds that an incident is resistant to 'A', but that this does not change over the 4 years.

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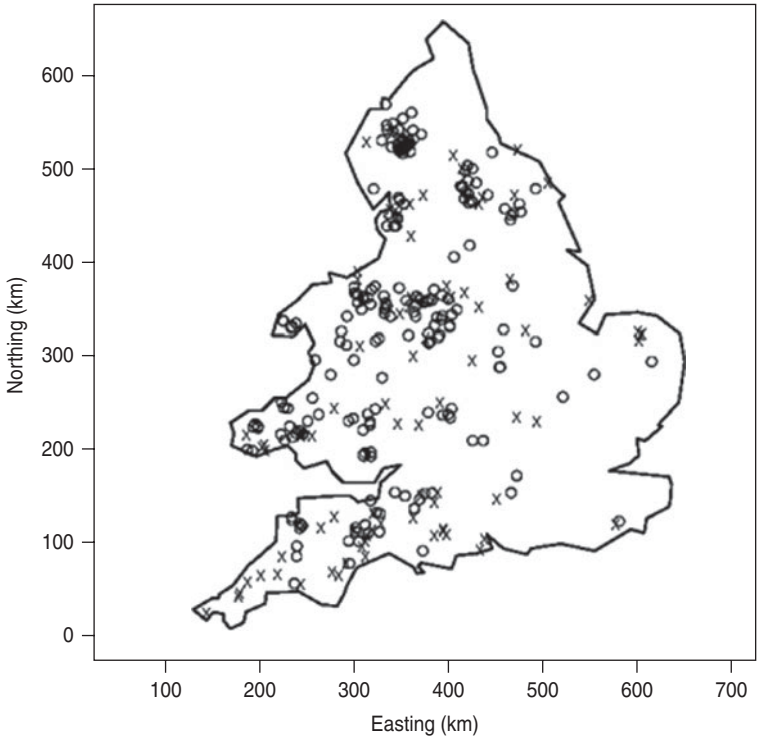

Fig. 2. Incidents of *S*. Typhimurium in cattle farms in England and Wales that were resistant to chloramphenicol between 1 January 2003 and 31 December 2006. Incidents with bacteria resistant to chloramphenicol are indicated by an open symbol (○), while incidents susceptible to chloramphenicol are indicated by a cross (×).

We model S as a Gaussian process with mean 0, and variance σ^2. This model allows the log-odds that *S*. Typhimurium incidents at two different farms are resistant to a given antimicrobial to be correlated. For a Gaussian process, at any given farm location x, the value of S, is a realization from a Gaussian distribution, i.e. $S(x) \sim N(0, \sigma^2)$. Both σ^2 and $S(x)$ are unknown, and are estimated during the model-fitting process.

The correlation between farms is assumed to decay exponentially with distance; for farms at locations x and x',

$$\mathrm{Cor}[S(x), S(x')] = \exp\{-\|x-x'\|/\phi\}. \quad (1)$$

Correlation of this kind allows $S(x)$ and $S(x')$ to be positively linked, with the link growing weaker as the distance between x and x' increases. The scale over which the spatial dependence tapers off is also estimated during the model-fitting process.

The correlation between the surface at each farm and any arbitrary point can be calculated using equation (1); we may thus characterize the distribution of the surface at, e.g. a fine grid of points across the country in terms of the values at the observation points, and hence provide a point estimate of the value and a measure of the uncertainty at each point on the grid. For more details of this process, which is known as kriging, see e.g. [25]. In the current context these kriging predictions make the most sense at points where there are cattle farms; at other points they simply indicate the likely variation in risk due to location that would occur if a cattle farm were to be situated there.

We used the package geoRglm in the R (www.r-project.org) environment, which employs Markov Chain Monte Carlo (MCMC) methods under the Bayesian paradigm. The Bayesian approach requires a description of prior beliefs about the model parameters. The choice for each prior is as follows.

(1) Covariate parameters β were assumed to be independent *a priori*, and each was given a vague Gaussian prior, $N(0, 10)$.
(2) The range parameter ϕ was given a discrete prior with possible values of 1 km, 2 km, …, 200 km, and prior probability proportional to $\exp[-\phi/20]$. This favours low ϕ values (i.e. short-range spatial correlation) and forces the data to assert the existence of any real spatial correlation.
(3) The variance σ^2 was given a scaled inverse χ^2 prior on 6 D.F. with scale value 1 (so that the expected value of $1/\sigma^2$ is 1). It is well known (e.g. [24, 25])

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that ϕ and σ^2 can be very strongly correlated *a posteriori* and that this can make individual identification of ϕ and σ^2 difficult. It is often only $\sigma/\phi^{1/2}$ which is well identified, but fortunately it is also this combination which is important in predicting the underlying surface $S(x)$. To avoid large, unnecessary values for σ^2 and poor mixing of the MCMC algorithm we truncated the prior, allowing only values of $\sigma^2 \leqslant 10$.

RESULTS

Choice of temporal covariates to be used in GLSM

In a GLSM analysis the temporal effect is considered through a set of time-varying covariates. We considered a number of possible temporal covariate effects for each antimicrobial, and for MDR, via the simple logistic regression GLM. The temporal covariate effects were: a linear trend, a quadratic trend, continuous piecewise linear interpolation between knots at each year end, sine and cosine terms for the annual cycle and for the bi-annual cycle. These models were compared using Akaike's Information Criterion. For sulphonamide compounds, chloramphenicol and MDR the best model included terms for both annual and bi-annual cycles. For streptomycin the best model included these terms and a continuous piecewise-linear trend in each year (this is similar to allowing for piecewise-constant year effects but removes the artificial jump that occurs in such models between 31 December of one year and 1 January of the next).

A GLSM was fitted for each individual antimicrobial (and MDR), and it is desirable that the spatial fields which are estimated for each antimicrobial be directly comparable so that we are able to determine any similarities. We therefore require the same temporal covariates for each antimicrobial, and so choose the covariate model with both annual and bi-annual cycles, and with continuous piecewise-linear trends in each year. The covariate terms in our model for the log-odds of resistance are therefore

$$\begin{aligned}\beta' f(t) = \beta_0 &+ \beta_1 \sin\left(\frac{2\pi t}{12}\right) + \beta_2 \cos\left(\frac{2\pi t}{12}\right) \\ &+ \beta_3 \sin\left(\frac{4\pi t}{12}\right) + \beta_4 \cos\left(\frac{4\pi t}{12}\right) + \beta_5 S03 \\ &+ \beta_6 S04 + \beta_7 S05 + \beta_8 S06,\end{aligned}$$

with a different set of βs for each antimicrobial (and MDR). Here $S03$, $S04$, $S05$, and $S06$ allow for the piecewise linear trend and are maximal at the knot points of 1 January 2003, 2004, 2005 and 2006, respectively.

Fitting the GLSMs

For each antimicrobial (and MDR) the MCMC algorithm was run for 20 million iterations, of which the first 0·5 million were treated as burn-in; to keep file sizes manageable, only one in every 800 iterations was actually stored. For all antimicrobials the chain mixed thoroughly for the temporal covariate parameters β^2, and for the scale parameter ϕ. Mixing for the variance σ^2 and for the more important quantity $w = \sigma^2/\phi$ was adequate for all four MCMC runs.

Table 2 shows the parameter estimates (posterior medians) together with a 95% credibility interval (CI) for each of the four model fits.

In terms of temporal effects, the most important terms (95% CI does not include zero) for streptomycin appear to be a bi-annual cycle and a peak in probability of resistance in the winter of 2004/2005. For sulphonamide compounds the most important terms involve the annual cycle, although the CIs for the coefficients of the bi-annual cycle only just include zero. The bi-annual cycle is also important for chloramphenicol, and one of the annual cycle terms only just includes zero. Finally the temporal MDR signal also appears to be mainly composed of an annual and bi-annual cycle. All of these results are consistent with the findings from the earlier exploratory GLM fits.

To visualize the temporal signals for each of the four fits we chose an 'average' point where the spatial signal $S = 0$. For each iteration of the (thinned) MCMC sample we then calculated the probability that an incident of *S*. Typhimurium would be resistant to antimicrobial '*A*' (or MDR). Figure 3 shows the median predicted value together with the 2·5th and 97·5th percentiles for each of the four model fits. These values are from the posterior predictive distribution of the temporal signal; the CI is not directly analogous to a confidence interval.

All four of the profiles showed a peak in the probability of resistance in mid to late spring, with a smaller peak in late autumn. The profiles for sulphonamide compounds, chloramphenicol, and MDR are very similar, all showing a consistent median level throughout the study period. The median level for streptomycin mirrored that of the other antimicrobials (and MDR) for the first 18 months before rising to a slight peak and then dropping off sharply

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Table 2. *Point estimates (median) and 95% credibility interval*

Parameter	Streptomycin	Sulphonamide	Chloramphenicol	Multidrug resistant
β_0	0·036 (−1·226 to 1·304)	1·470 (0·005 to 3·252)	0·922 (−0·335 to 2·307)	1·146 (−0·285 to 2·732)
*S*03	0·857 (−1·001 to 2·935)	−1·213 (−3·899 to 0·978)	0·125 (−1·847 to 2·063)	−0·317 (−2·585 to 1·831)
*S*04	0·841 (−0·629 to 2·448)	0·942 (−0·765 to 3·065)	−0·031 (−1·578 to 1·478)	0·330 (−1·330 to 2·149)
*S*05	1·497 (0·070 to 3·068)*	−0·271 (−2·178 to 1·390)	−0·227 (−1·783 to 1·154)	−0·101 (−1·815 to 1·492)
*S*06	−0·646 (−2·713 to 1·181)	−0·176 (−2·508 to 2·118)	−0·218 (−2·171 to 1·607)	−0·345 (−2·586 to 1·776)
$\sin(2\pi t/12)$	0·134 (−0·388 to 0·657)	0·626 (−0·002 to 1·419)	0·236 (−0·274 to 0·783)	0·305 (−0·276 to 0·958)
$\cos(2\pi t/12)$	−0·232 (−0·782 to 0·286)	−0·756 (−1·557 to −0·146)*	−0·419 (−0·965 to 0·100)	−0·689 (−1·389 to −0·115)*
$\sin(4\pi t/12)$	−0·556 (−1·134 to −0·080)*	−0·549 (−1·270 to 0·039)	−0·574 (−1·151 to −0·086)*	−0·603 (−1·274 to −0·058)*
$\cos(4\pi t/12)$	−0·149 (−0·660 to 0·339)	−0·280 (−0·980 to 0·325)	−0·147 (−0·675 to 0·341)	−0·225 (−0·867 to 0·336)
σ^2	1·517 (0·437 to 7·213)	2·574 (0·541 to 8·893)	1·401 (0·436 to 5·249)	2·239 (0·611 to 7·963)
ϕ	20 (6 to 62)	21 (8 to 57)	20 (6 to 72)	21 (9 to 58)
σ^2/ϕ	0·080 (0·011 to 0·676)	0·127 (0·015 to 0·622)	0·071 (0·009 to 0·501)	0·107 (0·015 to 0·560)

Credibility intervals marked with an asterisk (*) do not contain zero and therefore show either a high probability that the parameter is positive or a high probability that the parameter is negative.

over the final 18 months. This is consistent with Table 1 which shows that the proportion of positive results for streptomycin decreased to 49% in 2006 compared to 66% in 2003.

It is clear from Table 2 that there is a great deal of uncertainty in both spatial and temporal parameters. This is unsurprising given the paucity of data (294 binary observations), and indicates that any observed patterns should be treated with some caution, as the CIs will be large.

Figure 4 shows the posterior median estimate of the kriging surface S, the spatial contribution to the log-odds of an incident being resistant to a given antimicrobial. Each MCMC iteration supplies realizations from the joint posterior distribution of σ^2 and ϕ, and the value of the surface S at each data of the 294 data points. Values for S on a fine grid over England and Wales can then be estimated via ordinary kriging [25].

Patterns for the three antimicrobials and MDR show similarities, with higher odds in much of Wales, Wiltshire, and much of Devon, and with a further small peak around Leicestershire. All but the chloramphenicol plot also show an increased probability of resistance in North West England.

DISCUSSION

We analysed incidents of *S*. Typhimurium in cattle in England and Wales between 2003 and 2006. We looked for patterns in the spatial and temporal variability in the risk of antimicrobial resistance by fitting a GLSM to each of the four datasets. We focused on three antimicrobials (streptomycin, sulphonamide compounds, chloramphenicol) and MDR.

All four of the temporal profiles showed peak probability of resistance in mid-late spring and a lesser peak in late autumn. The mean signals for chloramphenicol, sulphonamide compounds, and MDR varied little from year to year, whereas that for streptomycin showed a sharp drop over the last 18 months. We are unsure why a sharp drop occurred in streptomycin as there were no obvious changes in farm management or laboratory procedures during 2005. This drop is surprising since streptomycin is linked to sulphonamide compounds in mode of resistance. One possibility is that it could have resulted from temporal changes in the dominant serotypes, which vary in their level of resistance [17, 26]. Indeed, variation in resistance prevalence has been related to

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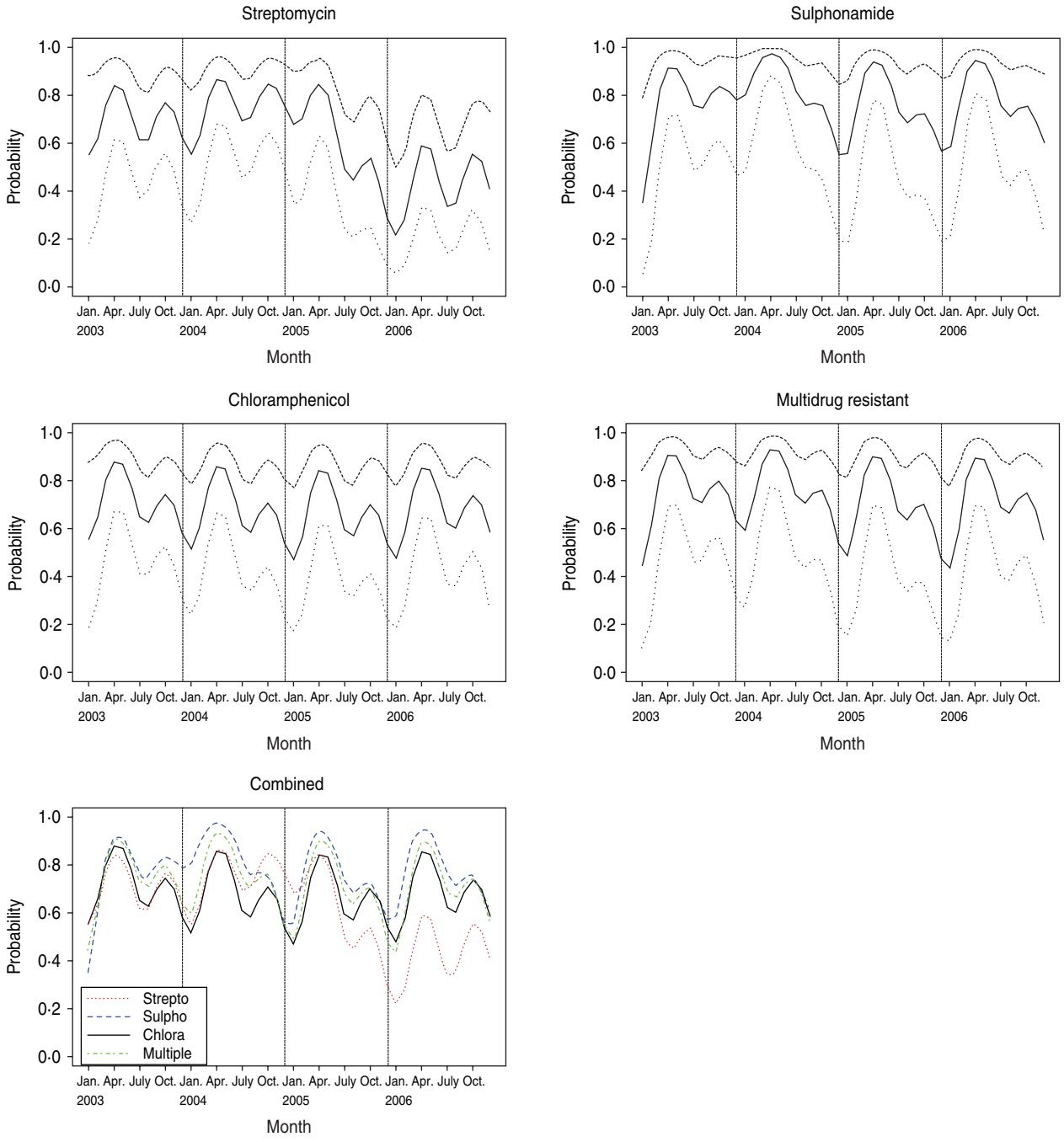

Fig. 3. Temporal signal for the probability of resistance for each of the four antimicrobials. Graphs show the median together with the 2·5% and 97·5% quantiles of $N \approx 25\,000$ Markov Chain Monte Carlo samples. The probabilities are calculated using the average value of the spatial signal, i.e. with $S(x) = 0$.

the clonal spread of particular strains as a result of husbandry and animal movement factors as well as to the variation in selective pressure exerted by antimicrobial usage [5].

It is possible that the levels of resistance that we recorded may be linked to antimicrobial usage since usage gives rise to selection pressure for resistance [11]. Sulphonamides and streptomycin are commonly used for treatment or as a prophylactic for respiratory and gastrointestinal infections in cattle [27] and the seasonal patterns that we observed may be associated with seasonal patterns of cattle management involving antimicrobial use.

We hypothesize a connection between the peaks in antimicrobial resistance in spring and autumn that we recorded and the strong seasonal patterns in cattle

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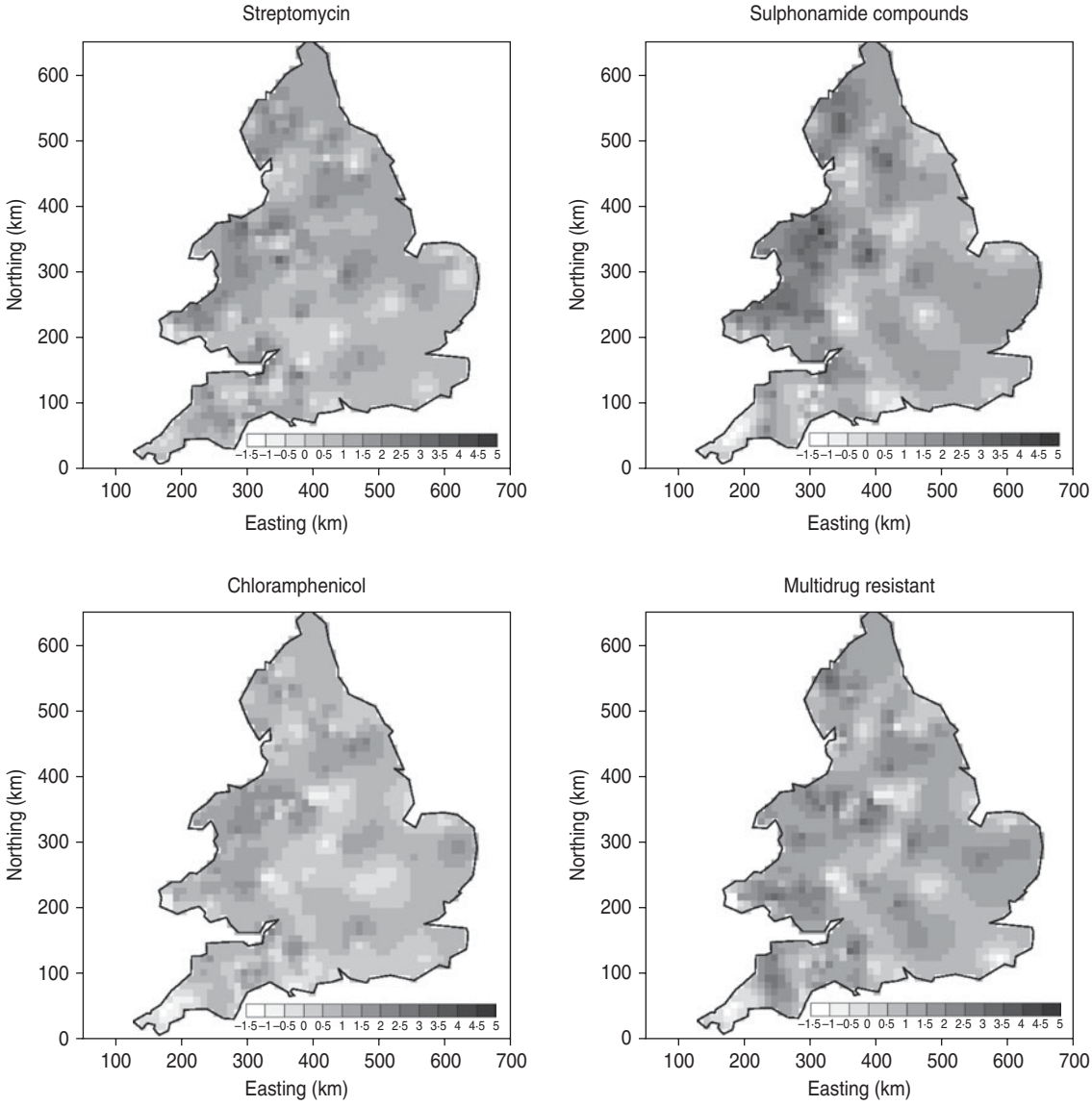

Fig. 4. Posterior median of the kriging surface *S*, the spatial contribution to the log-odds of an incident being resistant to a given antimicrobial, from 25 000 Markov Chain Monte Carlo samples.

births. Cattle births are characterized by a large spring peak and a smaller autumn peak [28]. Seasonal patterns occur in both dairy and beef cattle; beef cattle calving peaks in spring (with the largest monthly births in April) and troughs in winter (December), while dairy cattle calving peaks in autumn (August or September) and troughs around May [29]. At these times antimicrobials (e.g. sulphonamides) are administered (when calves are removed from the dam), to treat or prevent diarrhoea and pneumonia [30]. It has previously been noted that the selection pressure for antimicrobial resistance in *S*. Typhimurium is highest in calves due to the extensive use of antimicrobials in calf rearing and the type of *Salmonella* infection [14]. Outbreaks of salmonellosis in calves in Great Britain have often been caused by phage types that are characteristically multiresistant, e.g. definitive phage types (DTs) 29, 204 and 104 and have been linked to therapeutic antimicrobial use. They have also caused serious illness in the human population [14].

Seasonal patterns of adult cattle management involving antimicrobial usage could also play a part in seasonality of antimicrobial resistance. Antimicrobial usage and therefore selection pressure, tends to be high during the non-lactating phase for dairy cows and during cattle movement, and both of these activities peak in spring and autumn. Antimicrobials are

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routinely administered to entire adult cattle herds to prevent mastitis during the non-lactating period [30], which is commonly in late spring (2 months prior to calving). Prophylactic treatments are also typically used during transport; a high-risk period for infectious disease [30], which peaks strongly in spring and autumn (the autumn peak being the larger) [28]. Other contributors to the increase in risk of antimicrobial resistance during transport include the movement of carrier animals between herds and the assembly of susceptible animals in close confinement [30].

If the seasonal patterns described were the result of antimicrobial use, then this suggests a fairly rapid decrease in resistance at times when antimicrobial use in cattle is reduced. We found little evidence of rapid seasonal changes in antimicrobial resistance in the literature; however, quick response to the removal of antimicrobials is possible. There was a decrease in resistant bacteria in healthy animals and humans in the years immediately following the ban on animal antimicrobials as growth promoters in the European Union (EU) [31]. In *Enterococcus faecium* isolates from chickens and pigs, for example, resistance prevalence declined within 1 year of removal of growth promoters in many European countries. Indeed declines were seen within the first 3 months for four different antimicrobials. It was suggested that in the absence of selection pressure, a susceptible population began to replace phenotypically resistant strains [32].

Clearly antimicrobial consumption cannot explain the levels of resistance of chloramphenicol that we recorded because this substance has been banned from use in food-producing animals in the EU for many years. Co-resistance with other compounds is a likely explanation [33].

A spatial signal was visible for each antimicrobial, with certain attributes, such as high probabilities of antimicrobial resistance in North Wales shared between all four datasets.

Figure 1 demonstrated that North Wales has a very high density of farms with incidents of *S*. Typhimurium and it is possible that these two facts are related. Indeed some of the highest densities of cattle farms are located in Wales and the west of England. Beef cattle tend to be concentrated in the east and southwest of Wales and South West England, while dairy farms tend to be in southwest Wales, central England and the west coast, extending further north than beef farms [34]. Detailed information about the location of all cattle holdings was not available during our study. However, more recently, the density of registered premises in Great Britain by species and the number of incidents of *Salmonella* and have been reported [35] and confirm that *S*. Typhimurium aggregates around the Welsh borders and South West England. Despite the consistency of the spatial patterns across our four datasets, the credibility intervals (not shown) are large and so the trends should be viewed as tentative at best.

The seasonal patterns of resistance were similar for all antimicrobials. This is unsurprising since all three antimicrobials often occur in the characteristic multiresistant pentavalent resistance pattern (resistance to tetracycline, ampicillin, chloramphenicol, streptomycin and sulphonamide compounds) because they are chromosomally integrated as a single genetic island [23]. In brief, resistance can be exchanged between different bacteria by mobile genetic elements including plasmids, transposons, integrons and bacteriophages that carry genes for antimicrobial resistance. In some cases these elements may accumulate or co-integrate in a single host bacterium to give MDR [36]. MDR can then be transferred through one coherent piece of DNA (plasmid) that encodes specific resistance genes [37]. The pentavalent pattern has often been recorded in *Salmonella* isolates from dairy farms in England and Wales [23] and is common in *S*. Typhimurium, particularly definitive phage type (DT)104 [37]. This multiresistant phage type has caused numerous infections in food animals and humans in the UK and worldwide since the 1990s [15]. Not all resistances are carried on mobile genetic elements, as described above. In future, if sufficient data become available, it would be useful to compare other antimicrobials that are not linked in their mode of resistance, e.g. nalidixic acid. This analysis might demonstrate a different antimicrobial resistance response to local risk factors such as changes in antimicrobial usage.

In this work we do not specify any risk factor other than temporal covariates while studying patterns of antimicrobial resistance in *S*. Typhimurium. However, we can suggest potential risk factors for antimicrobial resistance, which have previously been identified for *Salmonella* infection that may act locally or regionally. Local risk factors include feed suppliers [38], herd size or composition (large farms and high stocking densities facilitate *Salmonella* dissemination) [16, 30, 39] and environmental contamination, e.g. bedding and water [17]. An increase in temperature in summer can increase the range of potential sources of

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contamination [17]. Herd-associated methods to reduce disease include purchasing replacement stock from direct sources rather than dealers, quarantine of purchased cattle for 4 weeks, housing sick animals in isolation areas and preventing wild birds access to cattle feed stores [40]. On a larger scale, studies have demonstrated contagious processes through farm-to-farm transmission. Risk factors acting on a regional scale include movement of contaminated humans or equipment between farms [30] the presence of suitable habitat for wildlife vectors [16, 41] or environmental factors such as runoff from pastures and wastewater contaminating local water and spreading between farms [20]. Infection can also be influenced by spatio-temporal factors. Infection in dairy herds, for example, is not constant over time and farms are more likely to become *Salmonella* positive if there are more positive farms within 30 km [21]. A more thorough (and necessarily more complex) analysis might try to gauge the extent of these effects by allowing for such factors explicitly. To begin with we suggest stratification of data, when sufficient is available, to assess the differences between dairy and beef herds. We also suggest incorporating trade relations (e.g. movement of cattle between farms) in the analysis. The vast majority of cattle movements occur over <50 km [42]; incorporating network parameters that account for the rapidly changing structure of the livestock industry would be very informative. Ultimately this could lead to the development of targeted surveillance and prevention measures aimed at reducing the incidence of antimicrobial resistance.

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DECLARATION OF INTEREST

None.

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