**Modelling the effects of livestock antibiotic usage on human foodborne disease**

**ABSTRACT**

Livestock antibiotic usage has been proposed as a major driver of antimicrobial resistance in human populations. This has contributed to the implementation of antibiotic stewardship programs aiming to curtail usage of livestock antibiotics. However, the possible consequences of livestock antibiotic curtailment on human health are poorly understood. In particular, the potential for increases in the carriage of foodborne pathogens in livestock due to a loss of antibiotic pressure, and subsequent increase in human foodborne disease. Here we use a mathematical model to explore the impact of curtailing livestock antibiotic usage on both antibiotic-sensitive and antibiotic-resistant foodborne disease in humans.

The model identified increases in overall human foodborne disease and a decrease in resistant human foodborne disease following livestock antibiotic curtailment. However, these effects can be mitigated through interventions to reduce animal-to-human transmission, and to a lesser extent, animal-to-animal transmission and the background rate of contamination in livestock. The magnitude of interventions needed to mitigate increases in human foodborne disease was found to vary across different case studies, suggesting that a “one-size fits all approach” across different agricultural settings, livestock hosts, and drug/bug combinations will likely not be efficacious or efficient.

This study provides a motivating example of one of many plausible scenarios following livestock antibiotic curtailment and identifies that even if increases in human foodborne disease are observed, existing agricultural biosecurity interventions can successfully mitigate the negative human health consequences of livestock antibiotic curtailment.

**INTRODUCTION**

Antimicrobial resistance (AMR) is currently one of the largest threats to human health, with a growing number of key antibiotic therapeutics being rendered ineffective by resistant bacterial pathogens. Livestock antibiotic usage has been identified as a potentially important driver of AMR in human populations, with cross-species transmission of resistant bacteria and resistance determinants potentially occurring at the livestock/human interface (**CITE**). This has led to calls to curtail the usage of livestock antibiotics, with legislature such as the 2006 European Union ban and 2017 US Food Drug Administration regulation on antibiotic growth promotion, aiming to safeguard the efficacy of clinical antibiotics and reduce the potential for transmission of resistant pathogens to human populations (**CITE**).

A range of beneficial outcomes have been reported as a consequence of livestock antibiotic curtailment, including decreased faecal *Enterococci* resistance rates in Denmark and Germany resulting from the 2006 growth promotion ban (**CITE**). Transient increases in the carriage of other resistant pathogens, increases in livestock carriage of foodborne pathogens and increases in therapeutic livestock antibiotic usage following antibiotic curtailment has also been identified in AMR literature (**CITE**). These negative consequences have been suggested to be attributable to increases in livestock production in the years following the European ban on antibiotic-mediated growth promotion and due to other resistance-related genetic factors (**CITE**). However, the unforeseen nature of these potential consequences highlights the risks of introducing substantial interventions into highly complex and poorly understood systems as part of a “precautionary principle” based approach (**CITE**). The need to better understand the potential long-term impacts of future AMR policy is also likely to increase in coming years, with new EU legislation strictly controlling the use of livestock antibiotics for metaphylaxis or prophylaxis by 2022 (**CITE**). However, the precise relationship between livestock antibiotic usage and antibiotic-resistant/sensitive human foodborne disease remains poorly understood.

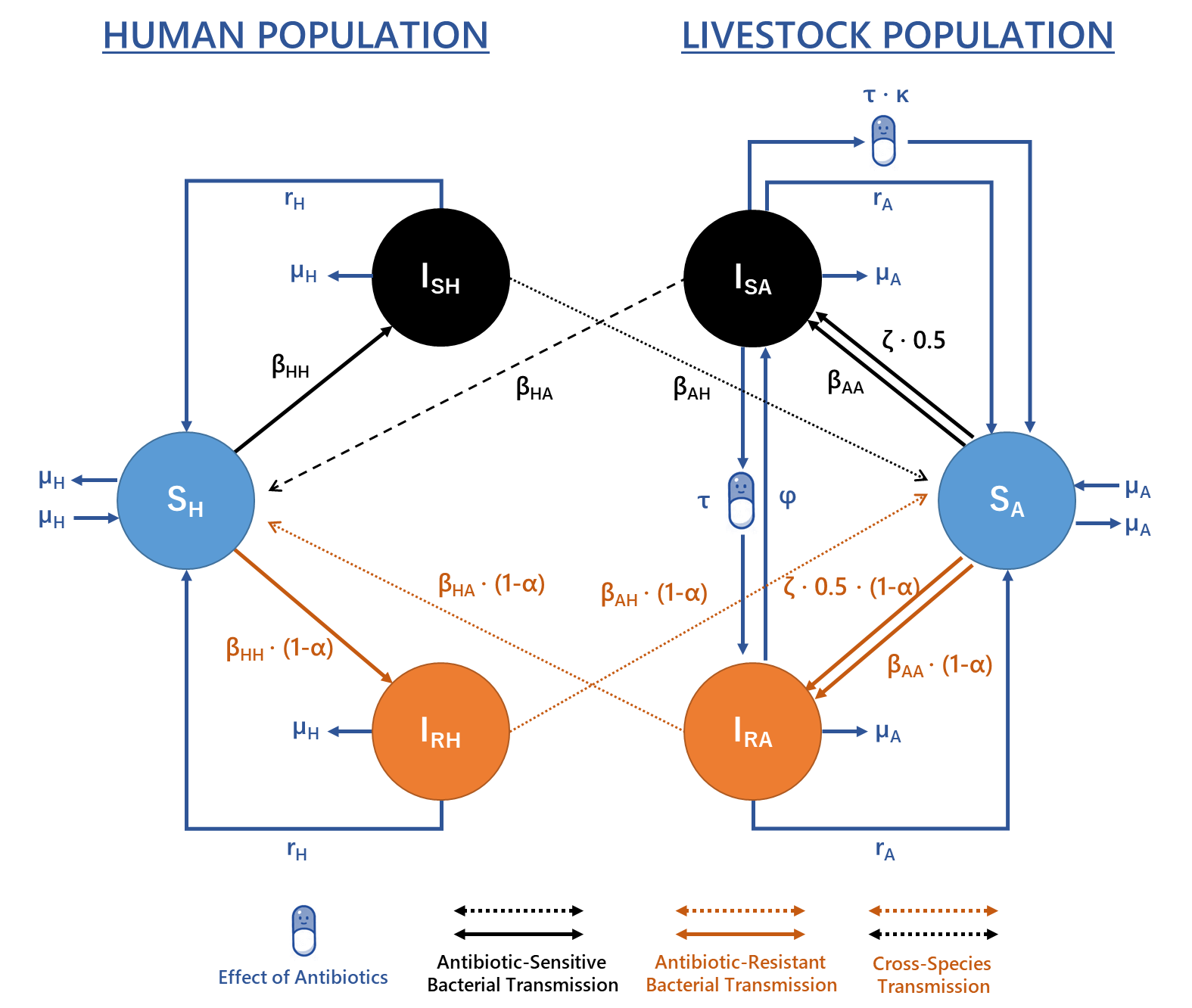
One approach to better understand the complexities of livestock antibiotic usage includes the use of mathematical models, which are simplified representations of complex real-world systems. These models can help by testing uncertainties, especially regarding the potential effects of livestock antibiotic usage on human health and the extent of AMR transmission at the livestock/human interface. However, there is a severe dearth of models which quantitatively explore these uncertainties (**CITE**). Existing frameworks include predictive risk assessment models and a small number of generalised deterministic models (**CITE**). Nevertheless, a significant number of knowledge gaps still exist, including a lack of understanding of the potential consequences resulting from livestock antibiotic curtailment and the impact of different mitigating scenarios on altering these outcomes.

To address some of the gaps in AMR modelling literature, a deterministic mathematical model was developed to explore the effects of livestock antibiotic curtailment on common foodborne infections in humans across a range of scenarios. By explicitly modelling both livestock/human populations and various assumptions regarding the effects of livestock antibiotic usage, we will explore the potential long-term consequences of livestock antibiotic curtailment, including alterations to the overall incidence of human foodborne disease and the antibiotic-resistant fraction of infections. Additionally, we will explore the effects and feasibility of introducing interventions to mitigate the potential negative consequences of livestock antibiotic curtailment.

**METHODOLOGY**

1. **Model Structure and Description**

A deterministic compartmental model was developed to describe the transmission of antibiotic-resistant and antibiotic-sensitive foodborne bacteria within and between livestock and human populations (**Figure 1**) (**CITE**). Each host population can be stratified based on their respective infection status: susceptible humans (SH), humans infected with antibiotic-sensitive bacteria (ISH), humans infected with antibiotic-resistant bacteria (IRH), susceptible livestock food-animals (SA), livestock food-animals infected with antibiotic-sensitive bacteria (ISA) and livestock food-animals infected with antibiotic-resistant bacteria (IRA).



**Figure 1 – Model structure describing the transmission of foodborne pathogens between/within livestock and human populations.** Model equations and parameters can be found described in the supplementary material (Table S2).

Transmission is simplified into four transmission routes: animal-to-animal (βAA), human-to-human (βHH), animal-to-human (βHA) and human-to-animal (βAH) transmission, with each *β* parameter linearly describing both indirect and direct transmission between compartments for model tractability. A background rate of transmission in the livestock population was also modelled (ζ), representing infection/contamination of livestock hosts from sources other than other livestock or humans. This generalised background transmission rate was scaled by a factor of 0.5 to ensure an equal influence of ζ on both antibiotic-sensitive and resistant transmission routes. Natural recovery from antibiotic-sensitive/resistant infection occurs in both human/livestock populations at rate rH and rArespectively. Per capita birth/death rates are represented by µA in livestock and µH in human populations.

We use a simplified parameter (τ) to describe the selective pressure and therapeutic effect of livestock antibiotic usage. We model the selective pressure of livestock antibiotics as a single transition rate, encompassing a range of evolutionary and biological phenomena that convert livestock between antibiotic-sensitive to resistant states. One plausible mechanism includes an implicit majority-minority relationship in each infected state, with livestock in each infected compartment possessing a small proportion of bacteria belonging to the other susceptibility class. Subsequent antibiotic usage may therefore remove antibiotic-sensitive bacteria (ISA) and allow the minority antibiotic-resistant strain to proliferate and dominate (IRA) (**Spicknall - CITE**). We similarly model a single reversion parameter (φ) to encompass a range of different biologically plausible phenomena that may cause reversion of antibiotic-resistant (IRA) to sensitive strains (ISA). This includes a relative fitness advantage of antibiotic-sensitive strains and the potential for resistant strains to gain compensatory mutations to reduce fitness costs (**CITE**).

We note that it was beyond the scope of this study to explicitly model these dynamics, rather we describe and acknowledge the *existence* of bidirectional resistance conversion though these simplified *φ and τ* parameters (**SPICKNALL CITE**). Additionally, we note that modelling within-host strain dynamics or dual-strain colonisation was beyond the scope of this study, therefore resistance was modelled as a binary phenomenon within both livestock and humans (**null neutral model - CITE**).

To reduce the linearity associated with livestock antibiotic usage on both livestock recovery and antibiotic-resistance conversion, a scaling parameter was introduced (κ) to model the relative efficacy of antibiotic mediated recovery in livestock. Transmission-related fitness costs associated with antibiotic-resistance were included and were assumed to reduce the rate of transmission for antibiotic-resistant bacteria as a scaling factor (α) (**CITE**).

Two primary outcome measures were considered in this study: 1) the daily incidence of human non-typhoidal salmonellosis per 100,000 population in the EU, defined as the sum of the incidence of antibiotic-sensitive and resistant infections at the long-term non-zero steady state. This required a scaling transformation to convert the model output daily proportion infected to the daily incidence infected using EU population data (SUPPLEMENTARY). 2) The fraction of antibiotic-resistant human non-typhoidal salmonellosis (I\*­RHProp) (defined as IRH / (ISH+IRH) at the long-term non-zero steady state. This quantity was directly calculated from the model output.

The long-term non-zero steady state of the two previously defined quantities was calculated using the “rootSolve” package, which dynamically solves the ODEs numerically until the calculated derivatives are determined to be at steady state. Although we note that it is likely that the current dynamics of AMR prevalence are in flux due to the influence of ongoing interventions, studying it at equilbrium is a useful indication of the long-term dynamics of the AMR and where the system is heading (**cite**). This is supported by temporal surveillance data which suggests that intervention-induced changes in the proportion of antibiotic resistance in populations tend to stabilise at a relatively constant levels in the absence of any further interventions (**cite**). This is especially the case for resistant *Salmonella* spp. infections, with a short duration of infectious human carriage (1/rH), facilitating a rapid approach to equilbrium.

1. **Case Study and Model Parameterisation**

An approximate Bayesian computation sequential Monte-Carlo (ABC-SMC) approach was used for parameter estimation. Summary statistics and distance functions were used to estimate the posterior probability distribution given the data, . Detailed methodology and pseudo-code of the ABC-SMC approach can be found in **Toni et al, (2009)**.

**Livestock Distance Function**

We note that while the primary outcome measures are relevant for humans (incidence and I\*­RHProp), the model also simulates the relationship between livestock antibiotic usage and the fraction of antibiotic-resistant livestock infection. We can fit this livestock portion of the model to surveillance data, to ensure that any modelled livestock interventions occur in a population with realistic dynamics. Four case studies were chosen to aid model parameterisation and to ground the model with EU epidemiological surveillance data.

These case studies were: 1) tetracycline-resistant non-typhoidal salmonella in fattening pigs to humans from 2015-2018 (**CITE**), 2) ampicillin-resistant non-typhoidal salmonella in fattening pigs to humans from 2015-2018 (**CITE**), 3) tetracycline-resistant non-typhoidal salmonella in broiler poultry to humans from 2014-2018 (**CITE**) and 4) ampicillin-resistant non-typhoidal salmonella in broiler poultry to humans from 2014-2018 (**CITE**) (**FIGURE**).

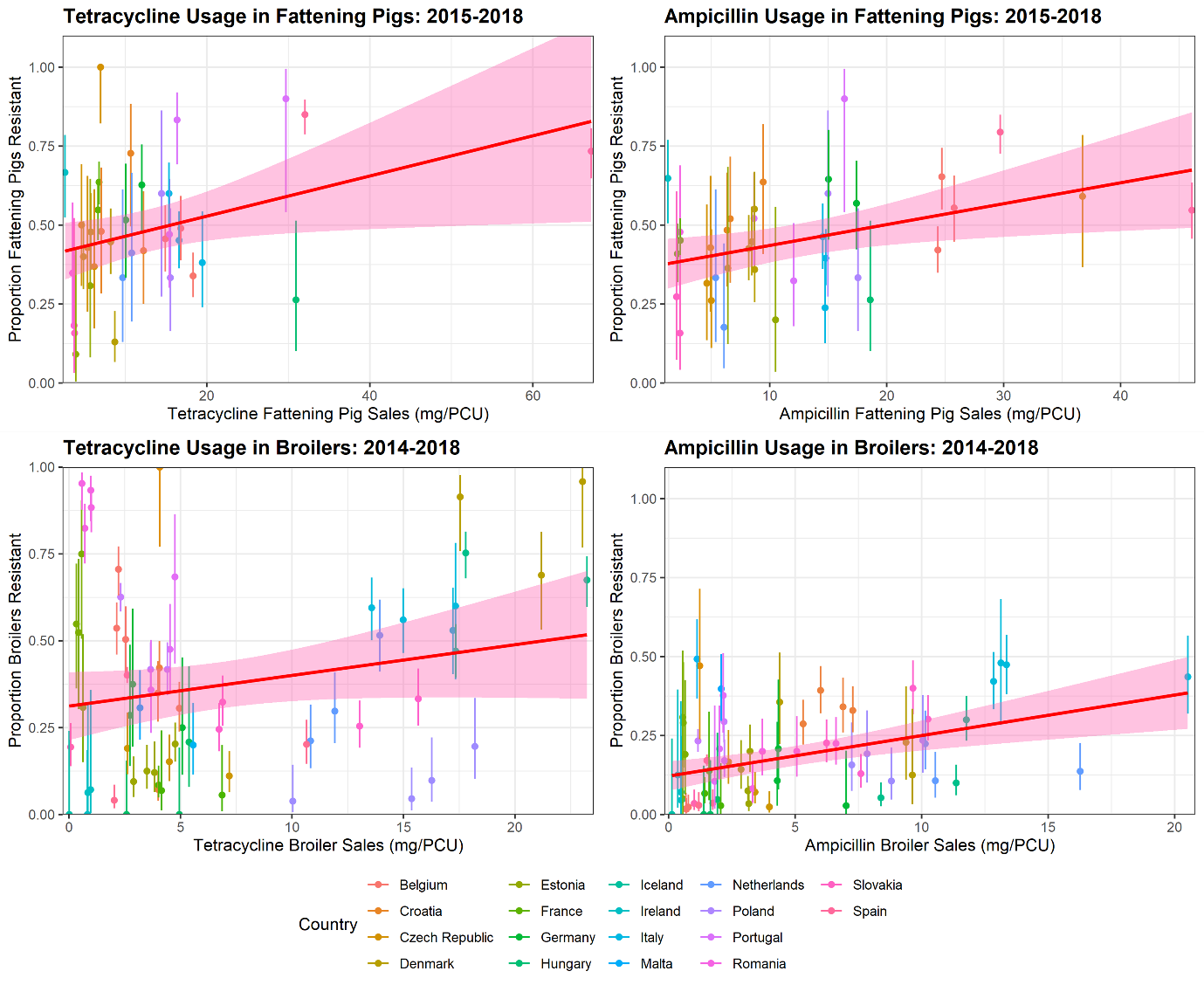


FIGURE x …. – NOTE WHICH ONES ARE SIGNIFICANT

These four case studies were chosen due to the high level of usage (both historical and current) of tetracycline and ampicillin in broiler poultry and fattening pigs, and the availability of resistance data for these two livestock species. We justify exploring the relationship between sales/usage and resistance for these four case studies as the basis for the model fitting and parameterisation in this study due to the observed statistically significant relationship between the two variables for three out of four included case studies, with one case study exhibiting a borderline significant relationship. We note that fluoroquinolone/azithromycin resistance have more clinical relevance for non-typhoidal salmonellosis due to their use to treat invasive NTS. However, we note that stewardship has already driven usage of these antibiotics in livestock to a low level (**CITE**). Therefore, it was not feasible to use these antibiotics as a case study for the impacts of livestock antibiotic curtailment.

Yearly ESVAC data for livestock antibiotic sales and EFSA data on the proportion of *Salmonella* spp. isolates obtained from livestock species resistant to the tetracycline/ampicillin were used to create usage/sales and livestock resistance pairs for each country in each respective case-study (**CITE**). These pairs spanned across multiple years for each country (**SUPPLEMENTARY MATERIAL**). Therefore, for any one country, there may be multiple usage/resistance pairs corresponding to different years in the dataset. These pairs were used to determine the observed relationship between livestock antibiotic sales/usage and the fraction of antibiotic-resistant livestock infection in European countries between 2014-2018/2014-2018 for each respective livestock species and was used for model parameterisation.

It is important to note that the stratification of each country into their respective yearly data for each data point introduces an assumption that the level of antibiotic usage will also be representative of resistance for a particular year. Due to the existence of lag between the effects of antibiotic stewardship interventions and alterations in either human or livestock resistance (cite), it is important to ensure that there is relative levels of stability in the yearly usage and resistance for each country. We note that for the majority of included countries, this temporal stability for each country across included yearly data points was observed (SUPPLEMENTARY).

A simulated dataset for each case study was generated by modelling the simulated fraction of antibiotic resistant livestock infections for each country/year observation, given the observed level of antibiotic sales for each country/year included in the dataset. A sum of squared errors distance function was then used to minimise the distance between the simulated () and observed () fraction of antibiotic-resistant livestock infection for each country/year data point. The number of country/year data points in each case study is denoted by .

ESVAC antibiotic sales data is found averaged for all livestock species in each country in the original surveillance report. A scaling calculation was therefore required to convert the generic antibiotic sales to a value specific to the modelled livestock host. Details of this can be found in the supplementary information. Note that due to a lack of accurate country-level antibiotic usage data, sales were assumed to be an accurate proxy for usage. Mentions of “usage” are therefore in reference to the ESVAC sales data. In accordance with the EFSA guidelines, countries with <10 isolates in the respective EFSA dataset for a particular year were omitted from the model fit to preserve the integrity of the dataset when fitting.

**Human summary statistics**

Two additional summary statistics were also used: 1) minimise the difference between the modelled daily EU incidence of human salmonellosis at baseline antibiotic usage and the observed ECDC daily EU incidence of human salmonellosis currently observed (0.593 per 100,000), 2) minimise the difference between the model estimated proportion of resistant human salmonellosis at baseline antibiotic usage and the EFSA averaged European proportion of resistant human salmonellosis specific for each case study. Baseline antibiotic usage for each case study was considered the unweighted average tetracycline/ampicillin sales across each included antibiotic country/year data point. 1) tetracycline-resistance in fattening pigs (**0.00491 g/PCU**), 2) ampicillin-resistance in fattening pigs (**0.00686 g/PCU**), 3) tetracycline-resistance in broiler poultry (**0.0125 g/PCU**) and 4) ampicillin-resistance in broiler poultry (**0.0131 g/PCU**).

**Fitted Parameters and ABC-SMC details**

The ABC-SMC approach was used estimate the marginal posterior probability distribution for six model parameters (θ) given the data, . Other model parameters were not fitted as estimates with high levels of certainty were available (rH, rA, μA and μH), or due to the relative nature of other transmission parameters with respect to βAA, βHA and ζ (βHH and βAH). These latter parameters were instead held at static values. Prior distributions for each fitted parameter can be found in the supplementary material (Table S3, Figure S2).

The ABC-SMC model fit was run for ten generations, with each generation running until the acceptance of 1000 particles. Acceptance thresholds (ε) can be found in thesupplementary material (Table S4). A multivariate normal distribution was chosen for the ABC-SMC perturbation kernel (**CITE**).

Mean point estimates from the approximated marginal posterior probability distributions of the tenth accepted generation were used as the final parameter sets for each respective case study. Point estimates and calculated 95% HDIs from the marginal posterior distribution for each model parameter can be found in the supplementary material (Table S2).

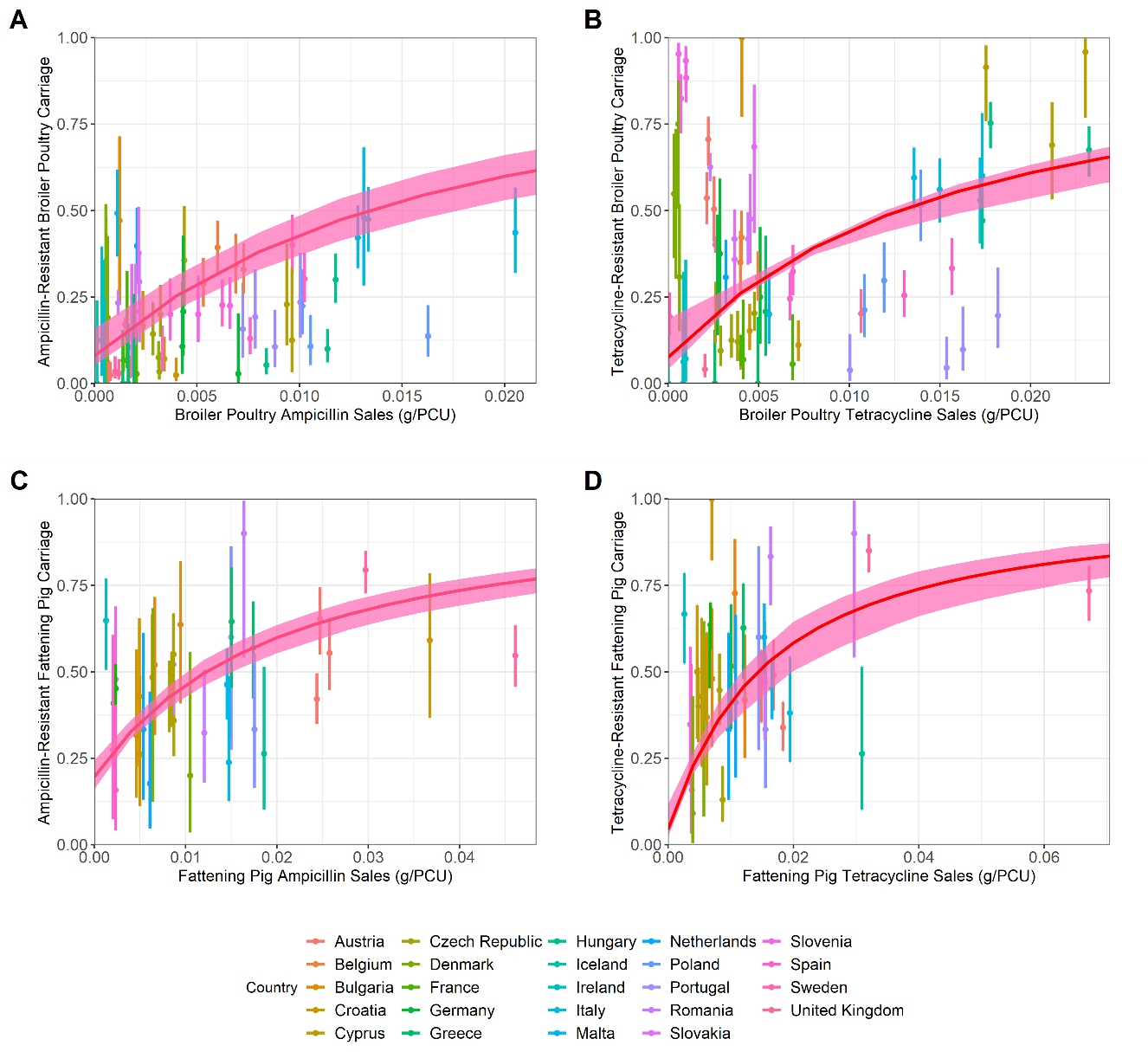
1. **Sensitivity Analysis**

A Fourier amplitude sensitivity test (FAST) approach was used to conduct a sensitivity analysis of the model system to the model parameters with regards to two outcome measures (**CITE**): 1) the daily incidence of human foodborne infection and 2) proportion of resistant human infection. The parameter space range chosen for the sensitivity analysis was limited to an order of magnitude above and below the parameterised values. For fitted model parameters, this range was taken as an order of magnitude above and below the mean of the fitted point estimate for each parameter across each considered case study.

The FAST approach was also used to identify the sensitivity of the model system to two intervention related outcome measures: 1) Relative changes in daily incidence when livestock antibiotics were curtailed (*τ* = 0 g/population correction unit (PCU)), compared to daily incidence at mean baseline livestock antibiotic usage across the four case studies (*τ* = 0.00934 g/PCU) and 2) Relative changes in daily incidence under antibiotic curtailment (0 g/PCU) relative to what is currently observed with current levels of antibiotic usage (0.593 per 100,000). An in-depth description of this sensitivity analysis can be found in the supplementary material.

**RESULTS**

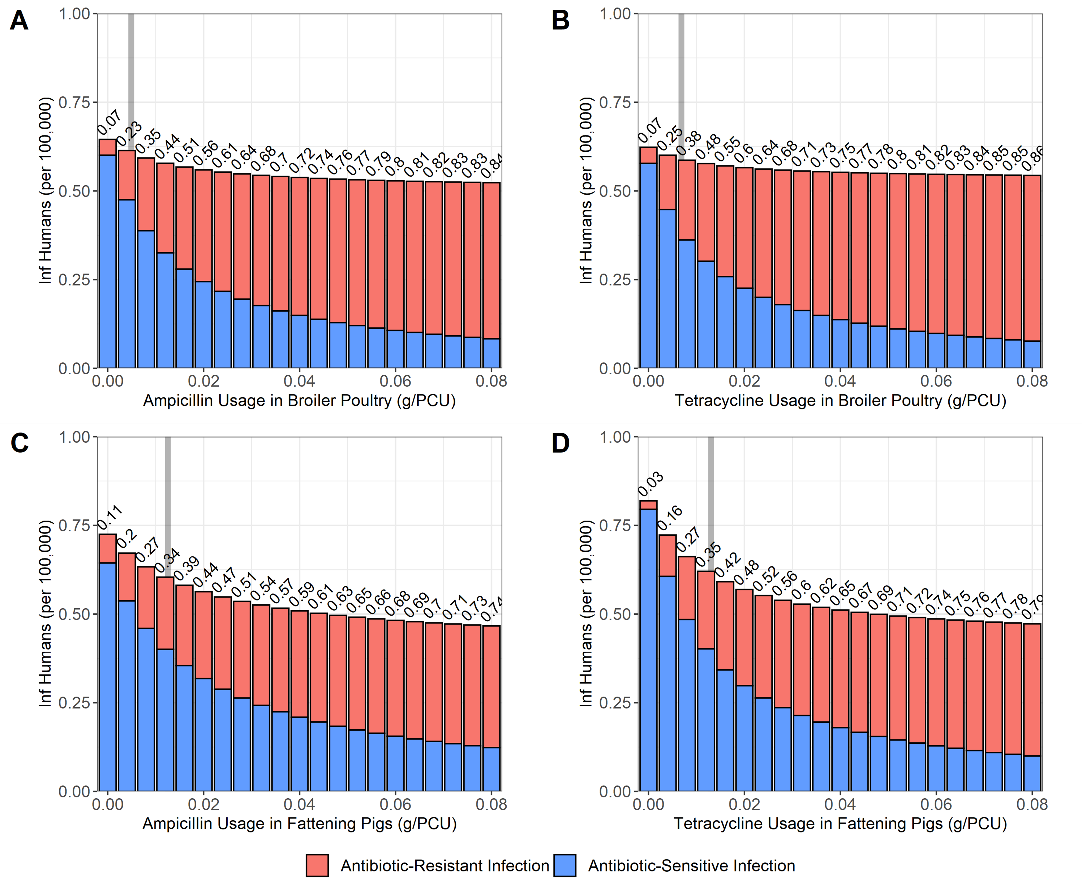
The relationship between observed country-level antibiotic usage data and livestock tetracycline/ampicillin-resistance surveillance data was plotted for all four case studies, with the model output overlaid, parameterised using ESVAC/EFSA data (Figure 2). It is important to note that the existence of the ζ parameter (ζ > 0) is necessary to prevent I\*RHProp decreasing to 0 upon livestock antibiotic curtailment. Inclusion of the ζ parameter was shown to provide a better fit to the model compared to a null model with ζ = 0(supplementary material; Figure S1). Approximated marginal posterior probability distributions for the fitted model parameters from the ABC-SMC approach can be found in the supplementary material (Figure S3).



**Figure 2 – Observed and estimated relationship between livestock antibiotic usage data and antimicrobial-resistant salmonellosis in humans. A) Ampicillin-resistance in broiler poultry, B) Tetracycline-resistance in broiler poultry, C) Ampicillin-resistance in fattening pigs and D) Tetracycline-resistance in fattening pigs.** Solid red lines and ribbons represent model fit resulting from the approximated posterior distribution using ABC-SMC and the corresponding 95% HDIs using the parameter point estimates from marginal posterior probability distribution. Country-specific 95% confidence intervals for the observed data (dots) were calculated for each case study using a 1-sample proportion test with continuity correction.

A Fourier amplitude sensitivity testing (FAST) approach was used to explore the sensitivity of the daily incidence of salmonellosis and I\*RHProp outcome measures to the model parameters. The most influential parameters for the daily incidence of salmonellosis were identified as the rate of animal-to-human transmission (βHA), with other parameters having a substantially reduced impact, and with I\*RHProp being most sensitive to transmission-related fitness costs (α), livestock antibiotic usage (τ), the efficacy of antibiotic-mediated recovery in livestock (κ) and antibiotic-resistant to antibiotic-sensitive reversion rate (φ) (Figure S4).

Using the fitted parameter values, the overall EU averaged daily incidence of human salmonellosis and the daily proportion of antibiotic-resistant human salmonellosis (I\*RHProp) was modelled at: 0.593 per 100,000 population and 0.35 for the ampicillin-resistant human salmonellosis from the broiler poultry case study at baseline ampicillin usage (τ = 0.0123 g/PCU). 0.593 per 100,000 population and 0.31 for the tetracycline-resistant human salmonellosis from the broiler poultry case study at baseline tetracycline usage (τ = 0.0116 g/PCU). 0.593 per 100,000 population and 0.31 for the ampicillin-resistant human salmonellosis from the fattening pigs case study at baseline ampicillin usage (τ = 0.0116 g/PCU). 0.593 per 100,000 population and 0.30 for the tetracycline-resistant human salmonellosis from the fattening pigs case study at baseline tetracycline usage (τ = 0.0067 g/PCU) (Figure 3).

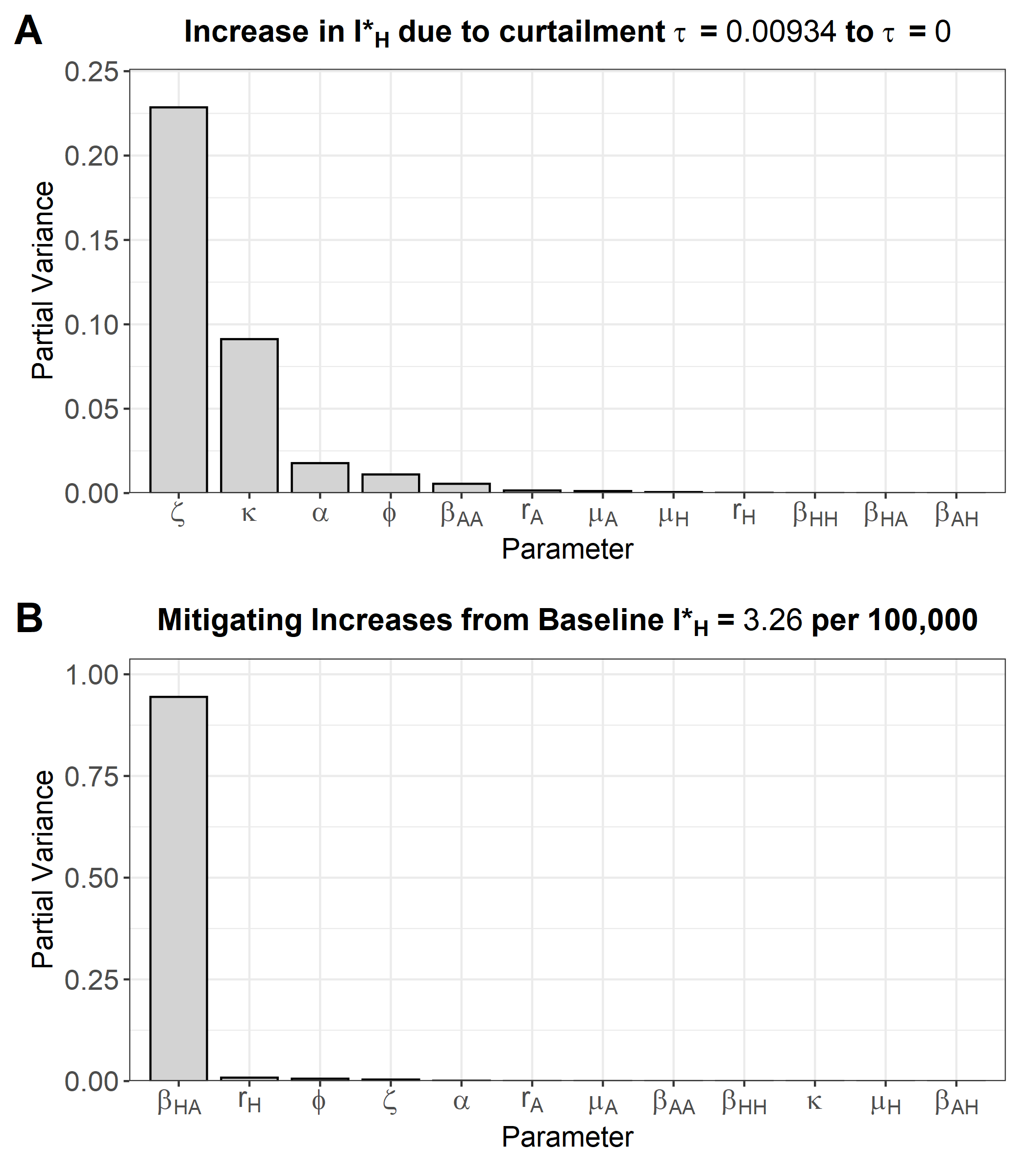


**Figure 3 – Impact of alterations in livestock antibiotic usage (τ) on the daily incidence of salmonellosis and the proportion of resistant human infection (**I\*RHProp**).** A) Ampicillin-resistant human salmonellosis from broiler poultry. B) Tetracycline-resistant human salmonellosis from broiler poultry. C) Ampicillin-resistant human salmonellosis from fattening pigs. D) Tetracycline-resistant human salmonellosis from fattening pigs. Grey bar denotes the case study specific baseline livestock antibiotic usage. Numbers above the bars denote I\*RHProp.

Curtailment of livestock antibiotic usage (τ → 0 g/PCU) resulted in small increases in the daily incidence relative to at baseline antibiotic usage levels across all case studies (Figure 3). Curtailment in the fattening pigs case studies resulted in the largest increase in the daily incidence with a X-fold (X per 100,000) increase relative to baseline levels, and an X-fold (X per 100,000) for the ampicillin and tetracycline case-studies respectively. Increases in the daily incidence for the broiler poultry case studies were relatively milder with an X-fold (X per 100,000) and an X-fold (X per 100,000) increase in the daily incidence for the ampicillin-usage tetracycline usage case studies respectively. Increases in livestock antibiotic usage above baseline usage levels in the three case-studies resulted in the opposite phenomenon being observed, with small decreases in overall human foodborne disease and increases in the proportion of resistant infection.

* **WILL NEED ANOTHER SUPPLEMENTARY PLOT WHICH SHOWS THE CHANGE IN PREVALENCE – NOT JUST INCIDENCE OF FOODBORNE DISEASE AS ANTIBIOTIC USAGE CHANGES.**

We next identified the parameters which had the greatest influence on relative increases in the daily incidence when livestock antibiotics were curtailed, compared to daily incidence at mean baseline livestock antibiotic usage across the four case studies (τ = 0.00934 g/PCU) (Figure 4A). Therefore, parameters that are identified as influential, are those that result in scenarios where curtailing livestock antibiotic usage has a greater relative increase in daily incidence. Daily incidence at mean baseline livestock antibiotic usage was allowed to vary and was not fixed across modelled parameter combination. The per capita rate of background transmission to livestock populations (ζ) and efficacy of antibiotic-mediated livestock recovery (κ) were found to be the most influential parameters in determining the relative increase in daily incidence from baseline livestock antibiotic usage when antibiotics where curtailed. (Figure 4A). Lower κ parameter values, and higher ζ parameter values resulted in lower relative increases in daily incidence when livestock antibiotics were curtailed (τ = 0 g/PCU) (Figure S5).

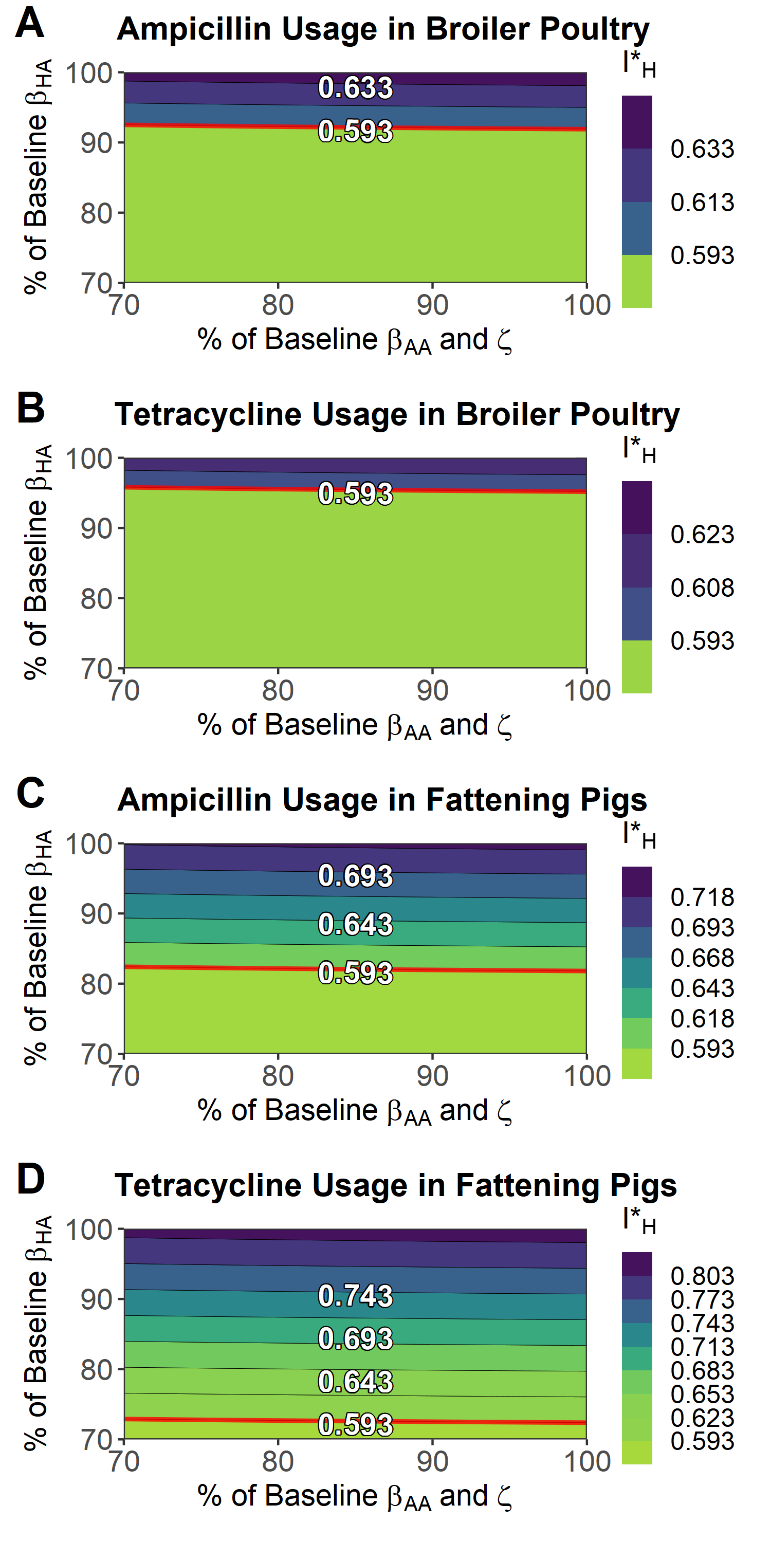


**Figure 4 – Fourier amplitude senstivity test (FAST) to identify the most influential model parameter for. Higher bars indicate greater sensitivity: A) The relative change in I\*H under curtailment (0 g/PCU) compared to the averaged baseline antibiotic usage level (0.0102 g/PCU). B) Mitigating changes in I\*H under curtailment compared to the level of foodborne disease experienced under current levels of livestock antibiotic usage (3.26 per 100,000 population).**

A sensitivity analysis was next performed to identify parameters that could best mitigate increases in I\*H under antibiotic curtailment (0 g/PCU) (Figure 4B). This was identified by curtailing livestock antibiotic usage and identifying model parameters that cause the greatest relative change in daily incidence from the *fixed* baseline value of 0.593 per 100,000 (the level of salmonellosis currently observed). By extension, interventions targeting these identified parameters will be more capable of reducing levels of daily incidence back down to baseline levels.

We identified the per capita rate of animal-to-animal transmission (βHA) as the key parameter to mitigate increases in daily incidence. Intuitively, decreasing βHA resulted in lower relative changes in daily incidence I\*H under antibiotic curtailment compared to 0.593 per 100,000. This therefore represents the best parameter to target to mitigate potential increases in daily incidence due to livestock antibiotic curtailment (Figure S6).

Alterations to βHA, βAA and ζ parameters were explored to identify potential interventions to mitigate increases in daily incidence under antibiotic curtailment (0 g/PCU), below a threshold of 0.593 per 100,000 population. This threshold represents a removal of livestock antibiotic selection pressure (0 g/pCU) and a prevention of increases in daily incidence above what is currently observed for human salmonellosis (0.593 per 100,000). Both βAA and ζ parameters were also explored as potential intervention targets, due to their relevance in agricultural biosecurity strategies to promote livestock health and mitigate livestock disease/AMR (**CITE**). A limited range of transmission parameter reductions were explored for each intervention scenarios.(0% - 30%) (Figure 5)**.**



**Figure 5 – Reductions to key model parameters, animal-to-human transmission (βHA), animal-to-animal transmission (βAA) and the background transmission rate to animal populations (ζ) to mitigate increases in the daily incidence of salmonellosis under livestock antibiotic curtailment (τ = 0 g/PCU). A) Ampicillin-resistance in broiler poultry, B) tetracycline-resistance in broiler poultry, C) ampicillin-resistance in fattening pigs and D) tetracycline-resistance in fattening pigs.** Axes represent interventions that reduce the labelled transmission rate(s) to % of their original values. Note that the top right corner of each contour plot represents a scenario with curtailment of antibiotics and no further alterations to any model parameter. The red line represents the threshold at which daily incidence is below current levels (0.593 per 100,000).

Only reductions to βHA were capable of mitigating increases to daily incidence below baseline levels across all considered case studies across the explored parameter space, with a reduction of X%, X % and X % required for each case study (Figure 5). Isolated or even combined reductions to βAA or ζ had a negligible effect on reducing daily incidence below baseline levels across any of the considered case studies. This corroborates the preceding sensitivity analysis suggesting that controlling animal-to-human transmission is vital to mitigate increases in daily incidence upon livestock antibiotic curtailment.

* ALSO MENTION WHAT HAPPENS TO THE PREVALENCE!!

**DISCUSSION**

A deterministic compartmental model was used to identify increases in the daily incidence of non-typhoidal human salmonellosis as well as decreases in the proportion of resistant human salmonellosis following livestock antibiotic curtailment. This was explored across four relevant antibiotic/livestock case studies. Only interventions to target animal-to-human transmission routes were found to mitigate the potential increases in human salmonellosis following livestock antibiotic curtailment.

It is possible that ongoing efforts to ensure farm-level and post-harvest biosecurity have already had tangible impacts on reducing βHA, βAA and ζ. These efforts include a greater awareness from agricultural/farm workers to provision clean feed, maintain clean livestock environs, prevent livestock overcrowding, reduce microbial contamination on carcasses, as well as comprehensive public information campaigns to promote safe handling of food products (**CITE**). Although the exact contribution of these interventions on transmission have yet to be quantified, current efforts to increase livestock wellbeing, agricultural biosecurity and farm-to-fork food safety are likely to be having an ongoing impact on preventing increases in human foodborne disease resulting from current livestock antibiotic stewardship interventions.

* Here maybe mention the effect on human prevalence aswell – not just incidence – so where ensuring livestock beta aa is also important

The ability to completely mitigate the negative human consequences of livestock antibiotic curtailment in the scenario-specific examples implemented in this study (Figure 5), also suggests that in certain cases, there may be the potential for improved biosecurity practices to replace livestock antibiotics as an alternative to prevent diseases of a livestock origin (**CITE – 3Rs**). However, further research is required to quantify the efficacy of these interventions on the specified transmission routes (**CITE**).

We note that there is currently no consensus in AMR literature regarding the definitive impact of antibiotic withdrawal, with this increase in foodborne disease in humans recognised in AMR literature as a hypothetical “worst case scenario” following antibiotic stewardship (**CITE**). However, by identifying that moderate strength biosecurity interventions are sufficient to control any detrimental human health effects following curtailment, if this “worst case scenario” is a reality, it is likely that the effects of this scenario could be mitigated.

We note that the key determinant of the increases in human foodborne disease following livestock antibiotic curtailment is the extent of antibiotic-mediated livestock recovery and transmission-related fitness costs (κ and α). As an illustrative example, preventing livestock antibiotic usage from enhancing the rate of clearance (κ = 0) and removing fitness costs (α = 0) prevents increases in livestock or consequently human foodborne disease following livestock antibiotic curtailment (Figure S7). This is consistent with several assumptions in veterinary AMR literature, which suggest limited human health effects following livestock antibiotic curtailment (**CITE**). Further experimental and epidemiological studies must be conducted to confirm the impact of sub-therapeutic and therapeutic antibiotic usage on the period of livestock infectious shedding and the impact of fitness costs of resistance on transmission potential.

* Basically nsay that we note the model doesn’t conform tto the concept of null neutrality – due to the existence of the zeta parameter
* But the fundamental model structure without this parameter is null neutral
* We tested the model zeta vs non zeta – and we find that the model without a forced intercept fits the data better
* We note that it was also beyond the scope of this study to model the phenomoneom
* So we decided the keep the non-null neutral structure – but we note that in future modelling we could use more compelx models with explicit modelling structures to sensure null neutrality.

We note that the existence of the ζ parameter prevents the model from being considered a neutral-null model (cite). However, a formal model comparison between the model with and without this parameter highlgihts that allowing the model to have an antibiotic usage level not zero when usage is zedro (zeta model) fitrs the datat better (supplementary material).

We also note that the existence

We note that the simplified system proposed in this study violates the concept of null-neutrality, a hypothesis originally conceived in evolutionary theory, and adapted in AMR modelling to prevent unrealistic assumptions when exploring the mechanisms driving strain co-existence dynamics (**50/50 STRAIN LIPSITCH - CITE**). This can be observed with the use of implicit assumptions to drive co-existence between strains (minority/majority of strains within compartments) and the presence of “immigration” infections (ζ) not tractable to the initial conditions at time 0. However, the absence of this in our model can be justified, as we reiterate that the aim of the model designed for this study was not to specifically characterise the evolutionary dynamics underlying coexistence. Rather the model was designed to implicitly acknowledge that this phenomenon exists, simplifying the mechanisms underlying coexistence and instead concentrating on the impact of host heterogeneity and zoonotic transmission on livestock AMR interventions. Nevertheless, as highlighted by Davies et al, (**CITE** – **both papers**) explicitly modelling mechanisms driving coexistence through models which conform to null-neutrality may have extensive implications on the potential impact of modelled interventions (**CITE**). Future modelling into livestock antibiotic curtailment may benefit from model comparison and exploratory modelling to assess the effect of assumptions, model structure and explicit modelling of biological phenomena on the impact of modelled interventions. – **MAKE THIS WHOLE SECTION A LOT SHORTER**

We also note that the effort required to achieve specific reductions across different transmission parameters is likely to be asymmetrical and non-linear. Quantifying the impact of livestock interventions on transmission through future epidemiological studies would likely be necessary for further studies which aim to predict the impact of livestock interventions. Additionally, future predictive modelling is still limited by a lack of understanding of effect of farm-to-fork food processing on microbial loads and resistance (**CITE**).

The differences observed across case studies could be attributable to heterogeneities in setting, livestock, host, pathogen and phenotypic resistance, with these differences being observed in AMR literature (CARMO ET AL). It is also plausible that the difference observed could also be due to the large level of “noise” present in the EFSA and ESVAC data used to fit the model. This “noise” could be due to several interacting factors: 1) livestock antibiotic usage and livestock resistance have a non-monotonic relationship, 2) livestock antibiotic sales are a poor proxy for usage, 3) available EFSA/ESVAC data is of insufficient quality or sample size to accurately capture the true relationship.

* We note that we attempted to overcome this using yearly data rather than aggregating data for each country

We note that bans of livestock antibiotics such as avoparcin have been followed by sharp decreases in the prevalence of antibiotic resistance, therefore such trends would likely be observed if the “true” relationship between livestock antibiotic usage and livestock resistance was observed (**CITE**). However, this assumes that variation in livestock antibiotic usage is the sole driver of differences between country-level resistance in livestock. Hierarchical statistical models should be used to explore country-level effects on the prevalence of livestock antibiotic usage (**CITE**). We also note that in the absence of granular, country specific antibiotic usage data stratified by antibiotic and livestock, use of antibiotic sales data as a proxy is the only alternative. In the absence of higher quality surveillance data in the future, model fitting might benefit from model-based inference of livestock antibiotic usage data. There is also a dearth of high-quality livestock datasets regarding carriage of foodborne pathogens, especially when compared to availability and size of human datasets (**CITE)**.

Due to the uncertainties with the data used in this study, it is important to note that the resulting model fit was not intended to predict the definitive consequences following alterations in livestock antibiotic usage. Rather the ABC-SMC approach was used to explore roughly parameterised case studies with the best available data, used as an illustrative example to explore the hypotheses and model structure proposed in this study. It is more useful to consider the parameterisation in this study as a method to ground the model across diverse parameter values, rather than an exact reflection of each case study. Despite the limitations of this data-driven modelling approach, we note that this is a significant improvement compared to an arbitrary parameterisation of the model system. Future improvements to AMR surveillance will likely improve the accuracy of future parameterisation of livestock AMR models (**CITE**).

This results from this study corroborates epidemiological surveillance and modelling studies, with decreases in antibiotic resistance following livestock antibiotic curtailment. However, we identify a potential increase in human foodborne disease following curtailment that may be completely mitigated through effective agricultural biosecurity interventions. The efficacy of these interventions suggests that a “one-health” attitude and an intensifying focus on improving livestock welfare to prevent human disease is critical when considering potential control strategies to tackle the AMR crisis.