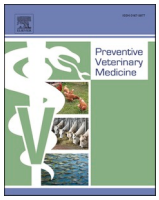
Preventive Veterinary Medicine 224 (2024) 106121

Contents lists available at ScienceDirect

Preventive Veterinary Medicine

journal homepage: www.elsevier.com/locate/prevetmed



Quantifying health risks from ESBL-producing *Escherichia coli* in Dutch broiler production chains and potential interventions using compartmental models

Minori Furusawa [a,b], Stefan Widgren [c,1], Eric G. Evers [d], Egil A.J. Fischer [a,*,2]

[a] Department of Population Health Sciences, Faculty of Veterinary Medicine, Utrecht University, the Netherlands
[b] Ministry of Agriculture, Forestry and Fisheries, Japan
[c] Department of Disease Control and Epidemiology, National Veterinary Institute, Sweden
[d] Centre for Infectious Disease Control (CIb), National Institute for Public Health and the Environment (RIVM), the Netherlands

ARTICLE INFO

Keywords:
Antimicrobial resistance
Chicken
Human health
Organic
Quantitative Microbial Risk Assessment
Simulation model

ABSTRACT

Extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* (*E. coli*) in animals are considered a human health threat, because this type of bacteria can serve as a reservoir of antibiotic resistant genes and act as a continuous threat of the emergence of new resistant bacteria, in addition to the direct effect of making infection untreatable. Although the prevalence of ESBL producing bacteria in broilers was drastically reduced in the Netherlands, chicken meat still has the highest prevalence among meat products. Therefore, further control of the ESBL-producing *E. coli* in the broiler production chain is important to reduce public health risks. The main objectives of this study were to evaluate the effectiveness of intervention scenarios to reduce the transmission of ESBL-producing *E. coli* in the broiler production chain and to quantitatively estimate the risk to public health. In this study, we developed two different types of transmission models that described the observed time-related decline in prevalence during a production round: one with time-dependent decline in susceptibility and one with partial immunity to phylogenetic groups. Both models incorporated the environmental contamination effect between production rounds and within flocks. The parameter values, including transmission rate and recovery rate, were estimated by Approximate Bayesian computation (ABC) method using data from a longitudinal study in a Dutch organic broiler farm. We applied the models to the three production stages in the broiler production chain, beginning from the Parent Stock (PS) farms, the hatcheries, and to the broiler farms. In our models, eggs were collected from different parent stock farms and transported to the hatchery and from there to a broiler farm. The size of a flock and the number of farms were adjusted to the Dutch situation. Both models were able to describe the observed dynamics within and between the production stages equally well, with estimated ESBL-producing *E. coli* prevalence of 8.98% and 11.47% in broilers at slaughter and 0.12% and 0.15% in humans due to chicken consumption. Both models indicated that improving farm management to eliminate the bacteria from the environment was the most effective intervention, making this outcome robust. Although chicken meat consumption is not a major risk factor for human carriage of the bacteria according to our models, reducing the bacteria in the PS and broiler farm environment to at least one percent can further decrease the prevalence in humans.

1. Introduction

Extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* (*E. coli*) produce enzymes that inactivate beta-lactams, first- to third-generation cephalosporins, and aztreonam, which are widely used antimicrobials to treat infections in both human and veterinary medicine (Chong et al., 2011; Mevius et al., 2018). In addition, the bacteria in animals can serve as a reservoir of antibiotic resistant genes. The

* Correspondence to: Yalelaan 7, 3584CL Utrecht, the Netherlands.
E-mail address: e.a.j.fischer@uu.nl (E.A.J. Fischer).
[1] https://orcid.org/0000-0001-5745-2284
[2] https://orcid.org/0000-0002-0599-701X

https://doi.org/10.1016/j.prevetmed.2024.106121
Received 16 January 2023; Received in revised form 28 December 2023; Accepted 10 January 2024
Available online 19 January 2024

prevalence of ESBL-producing *E. coli* in the general Dutch population was reported to be around 5.0% to 8.6% (Reuland et al., 2016; van den Bunt et al., 2019). Although chicken meat accounted for only 4.5% of intestinal carriage of ESBL or pAmpC gene in the general human population and human carriage of such bacteria was mainly attributed to human sources (Mughini-Gras et al., 2019) and human activities such as previous antimicrobial treatment and international travel (Chong et al., 2011; Pitout, 2009), the bacteria in animals are still a risk to public health. ESBL-producing bacteria can act not only as an infectious agent but also serve as a continuous threat of the emergence of new resistant bacteria (EFSA and ECDC, 2022). The genes coding for ESBL production can be transferred to other bacteria species by conjugation, which can eventually cause beta-lactam resistance in many bacteria species (Mevius et al., 2018).

In the EU, monitoring of the ESBL-producing bacteria in livestock has been carried out yearly based on Commission Implementing Decision 2013/652/EU to track the possible source of emerging resistant bacteria (EFSA and ECDC, 2022). Prevalence of presumptive ESBL-producing *E. coli* isolates from broilers ranged between 27.6% and 29.7% in 2020 in European countries. In the Netherlands, broilers, had the highest prevalence of ESBL-producing *E. coli* in 2014 at around 66% (Nethmap-MARAN, 2021). However, by setting reduction targets on antimicrobial use in animals and restricting the use of ceftiofur in hatcheries (Mevius and Heederik, 2014; Nethmap-MARAN, 2019), the prevalence of animals at slaughter was drastically reduced to 9.8% in 2020. On the other hand, at the consumption level, chicken meat still has the highest prevalence (9%) among meat products and further control of the ESBL-producing *E. coli* in the broiler production chain is important to reduce public health risks.

The Dutch broiler production consists of several stages, and ESBL-producing *E. coli* are detected at every level of the production chain (Dame-Korevaar, 2020; Dierikx et al., 2013). ESBL-producing *E. coli* were observed in as young as two-day-old chicks on Grandparents Stock (GPS) farms and in one-day-old chicks on Parent Stock (PS) farms (Dierikx et al., 2013). In hatcheries, the prevalence of eggs ranged from 0% (Oikarainen et al., 2019) to 3.8% (Mezhoud et al., 2016) and ESBL-producing *E. coli* can be found in as young as one-day-old chicks in broiler farms (Dierikx et al., 2013; Huijbers et al., 2016; Laube et al., 2013). In a longitudinal study, the prevalence in broilers on the day of arrival at the farm was about 30% and then increased to as high as 100% on day 3 (Huijbers et al., 2016). Then it decreased to 20% on day 42 and went up to 40% on day 70. A similar trend was also reported in an experimental study in PS birds (Dame-Korevaar et al., 2017); the prevalence began at around 90% on day 7 and started to decrease in week 11 from 46% to finally 1% in week 19. Most ESBL-producing *E. coli* isolates are obtained from healthy animals and generally have little implication for hosts' health (Kuhnke, 2020).

Improvements in biosecurity and hygiene management, disinfection of eggs, and vaccination have been implemented in poultry farms to reduce the prevalence of *E. coli*, including those that produce ESBL (Becker et al., 2021; Hao et al., 2013; Luyckx et al., 2015a; Luyckx et al., 2015b; Mo et al., 2016; Motola et al., 2020; Sadeghi et al., 2018; Swelum et al., 2021). Competitive exclusion (CE) is a method to protect chicks from undesirable bacteria, including *Salmonella*, by feeding intestinal microbiota from healthy Chickens. The effects of CE on ESBL-producing *E. coli* were studied in experimental settings and considered to be useful to reduce transmission and prevent colonization, especially when applied to young chicks for several days (Ceccarelli et al., 2017; Dame-Korevaar et al., 2020a; Dame-Korevaar et al., 2020b; Methner et al., 2019; Methner and Rösler, 2020; Nuotio et al., 2013). Currently, inactivated vaccine (Nobilis E.coli) and live attenuated vaccine (Poulvac E. coli) are used to protect chickens from *E. coli* (Swelum et al., 2021).

Since large-scale intervention studies in the broiler production chain are difficult to carry out due to cost and practical issues, several mathematical models were developed to simulate the transmission and assess the effectiveness of interventions (Dekker, 2019; Huijbers et al., 2016; Plaza-Rodríguez et al., 2018). Plaza-Rodriguez et al. (2018) incorporated several production stages and transportation effects into their transmission model and showed a difference between animal and flock transmission dynamics. However, they did not consider the effect of mixing birds and eggs from different origins, or the actual size of a flock and number of flocks. In their study, transmission parameters were set at a constant value, although several studies have reported fluctuation of the ESBL-producing *E. coli* prevalence as a function of birds age (Apostolakos et al., 2019; Dame-Korevaar et al., 2017; Dierikx et al., 2013; Huijbers et al., 2016; Laube et al., 2013). These age-related declines in the prevalence are possibly due to a change in susceptibility caused by shifts in the microbiota of chickens (Diarra et al., 2007) or changes in phylogenetic groups of the bacteria (Apostolakos et al., 2019).

The main objectives of this study were to evaluate the effectiveness of the intervention scenarios to control the transmission of ESBL-producing *E. coli* in the broiler production chain and to estimate the risk to public health. Most importantly, we developed and parameterized two different types of within-flock transmission models, one with the age-related decline in susceptibility and another with partial immunity to phylogenetic groups. We further incorporated the environmental contamination effect between production rounds and within flocks.

2. Material and methods

2.1. Movement between the production stages

To study the transmission in a broiler production chain, multiple production stages were connected in one model. In general, the production chain starts with the import of Grandparents Stock (GPS) chicks. Their offspring become the Parent Stock (PS) and are raised to lay eggs on parent farms. Then, PS-produced eggs are transported and incubated in hatcheries. Finally, the hatched chicks are transported to the broiler farms where they are reared until slaughter. In this study, the latter three production stages were considered: PS farms (n = 195), broiler hatchery farms (n = 13), and broiler production farms (n = 637) (Fig. 1). The number of farms reflects the Dutch situation (CBS Statline 2022a; CBS Statline 2022b; Ellen et al., 2012).

The movement of animals and eggs from one production stage to another was done by scheduling the demographic and movement events to modify the state of a production stage at a pre-defined time. In the model, four types of poultry movements were defined: enter, internal transfer, external transfer, and exit.

The enter events added new PS birds to a production stage. In this study, around 2000 18-week-old PS birds were brought to a PS farm every 66 days by an enter event where 10% of them are infected with ESBL-producing *E. coli* (the numbers were randomly generated from a binomial distribution and rounded, thus were not exactly 2000) (Apostolakos et al., 2019; Dame-Korevaar et al., 2017; EFSA Panel on Animal Health and Welfare, 2010). Then, by an internal transfer event, the PS chicks changed the age category five times with a 66-day interval. After finishing the fifth category, when the chick was around 65 weeks old (reared for 47 weeks on a PS farm after arriving at 18 weeks of age), it was removed by an exit event. In short, a PS farm consisted of five different age categories with 2000 birds per category and a PS bird had contact with all other PS birds on the farm. Eggs were laid at a rate of 0.065 per day (b) at PS farms and transported daily to hatcheries through an external transfer event. The laying rate was intentionally set lower than reality to maintain a stable population size at broiler farms. In real broiler production, not all eggs are sent to the hatcheries; some are exported or discarded. We incorporated this by low laying rate in our model. After 20 days of incubation (hatching rate (δ)), the hatched chicks were transported to broiler farms by an external transfer event (Archer and Lee Cartwright, 2017). After 42 days of the rearing period, the broilers were slaughtered through an exit event (EFSA Panel on Animal Health and Welfare, 2010). External transfer events moved eggs

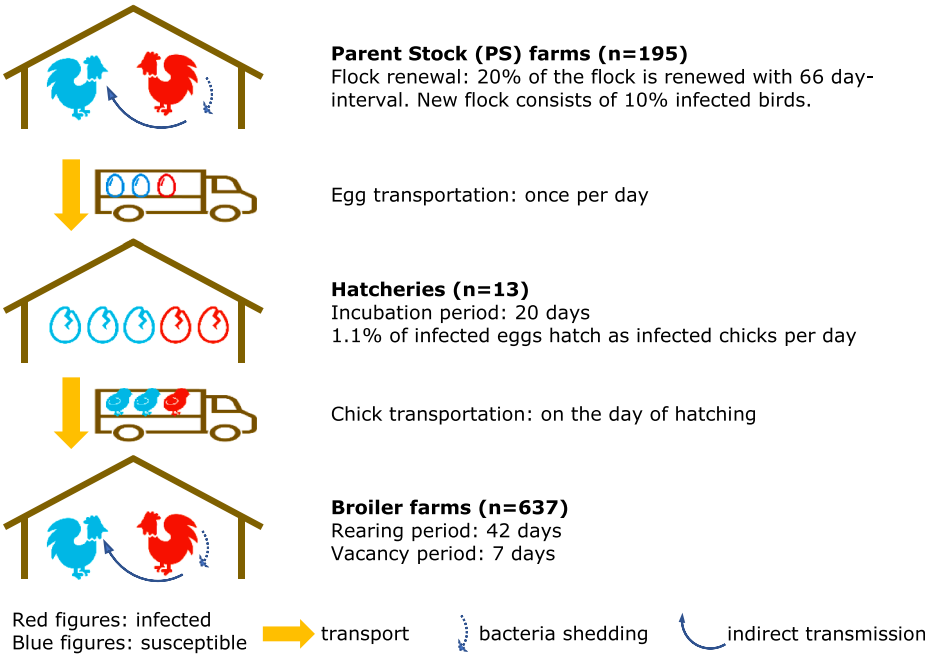


Fig. 1. Overview of the broiler production chain and transmission routes. Between and within transmission routes of Extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* in the three production stages of the broiler production pyramid model.

and chicks from one production stage to another. Finally, exit events removed PS birds and broilers from the stage to slaughter.

2.2. Transmission between the production stages

Transmission between production stages occurred by transporting infected animals/eggs, and transmission within the PS and broiler farms occurred indirectly through the contaminated environment.

In hatcheries, hatchlings are thought to become infected from contaminated eggshells, known as pseudo-vertical transmission (Mezhoud et al., 2016; Oikarainen et al., 2019; Projahn et al., 2017). Contaminated eggs were produced by infected PS birds at a certain proportion while all eggs from susceptible PS birds were assumed to be uncontaminated. Projahn et al. (2017) reported that one egg out of 280 eggs was positive for ESBL-/pAmpC-producing enterobacteria on the outer surface after disinfection, therefore, we assumed that pseudo-vertical transmission (l) occurred at a rate of 0.0036. The same study reported that three of the 280 hatchlings were positive for enterobacteria although none of them were positive for ESBL-/-pAmpC-producing enterobacteria. We used the data of the enterobacteria positive eggs for a worst case scenario and set the hatching colonization rate (m)) at 0.011, meaning that 1.1% of the chicks hatched as infected from the contaminated eggs per day (Projahn et al., 2017).

In broiler farms, bacteria can be transmitted from infected chicks from the hatcheries and indirectly via the remaining environmental contamination from the previous production round (Dierikx et al., 2013). The broiler farms used an all-in-all-out system with a seven-day interval, meaning that all birds on a farm were of the same age (Marshall, 2018). We assumed the daily death rate to be 0 as we wanted to ignore the effects from the changes in population size but to focus solely on the transmission dynamics. The time of the movements and the destination farms were fixed deterministically, but the numbers of moved birds and eggs were chosen stochastically. All values related to population dynamics and transmission between the production stages are summarized in Table 1.

2.2.1. Transmission within a flock (PS farms and broiler farms)

We used two different stochastic compartment models as the basis of within-flock transmission: the susceptible-infected-susceptible (SIS) model (Fig. 2a) and the susceptible-infected-susceptible-infected-recovered (SISIR) model (Fig. 2b). We decided to use these two models because other models were unlikely to describe the transmission dynamics. For example, the SI model, in which an infected bird stays infected for the rest of its life, or the SIR model, in which a recovered bird from a single infection acquires immunity, was not suitable, given the trends in prevalence observed in several studies (Dame-Korevaar et al., 2017; Dierikx et al., 2013; Huijbers et al., 2016; Laube et al., 2013). Latent period was not included in the model because it is short as the excretion of the bacteria begins within 24 h after inoculation

Table 1
Parameters and values for population dynamics and transmission in hatcheries in the SIS and SISIR models.

Notation	Value	References
Duration in Parent Stock farms	47 weeks	Production period: 18-22 to 60-65 weeks of age (EFSA 2010)
Interval of population renewal in Parent Stock farms	66 days	Five age classes in 47 weeks (approx. 330 days) on Parent Stock farm
Proportion of renewal of Parent Stock birds	20%	3,000-8000 birds per group, 10,000-30,000 birds per farm. (EFSA 2010)
Proportion of infected birds in renewed Parent Stock birds	10%	3 to 20% (Apostolakos et al., 2019; Dame-Korevaar et al., 2017)
Duration in hatcheries (incubation period, Hatching rate (δ))	20 days (0.05)	21 days (Archer & Cartwright, 2017)
Duration in broiler farms	42 days	42 days (EFSA 2010)
Interval between broiler production rounds	7 days	7 days (Marshall, 2018)
Laying rate (b)	0.065	see text
Daily death rate (i)	0	see text
Pseudo vertical transmission rate (l)	0.0036	0.0036 (95% CI: 5.81 $\times 10^{-6}$, 0.014) (Projahn et al., 2017)
Hatching colonization rate (m)	0.011	0.011 (95% CI: 0.0018, 0.025) (Projahn et al., 2017)

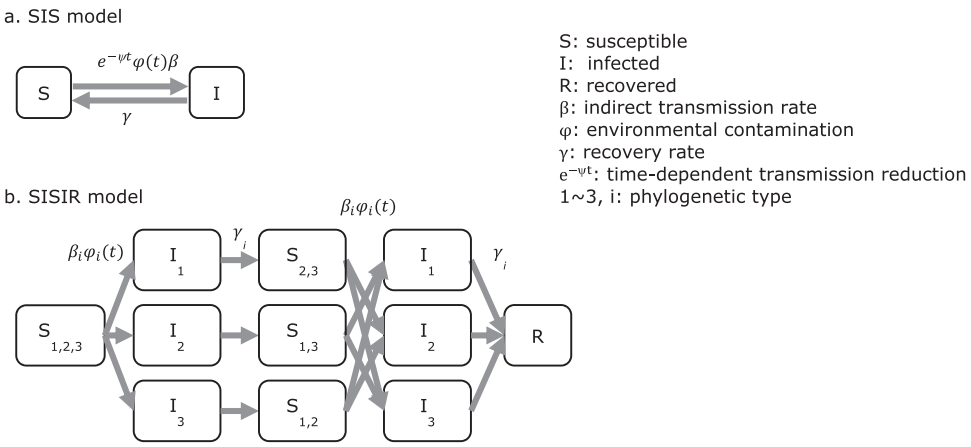


Fig. 2. Compartment models. a. SIS model with time-dependent transmission reduction. Transmission can occur indirectly. b. SISIR model with three phylogenetic groups. A susceptible bird (S_i) becomes infected with one of the three phylogenetic types and becomes an infected bird (I_i). After recovery from the first infection, the bird becomes susceptible again and can acquire another phylogenetic type. After two infections, the bird becomes immune to the bacteria (R). Transmission occurs indirectly without time-dependent transmission reduction.

(Ceccarelli et al., 2017; Dame-Korevaar et al., 2019,). Although the main transmission route is still unknown (Dame-Korevaar et al., 2019), we expected that indirect transmission from the faeces and the environment as the main source of infection, therefore included only indirect transmission route in the models.

In the SIS model, a susceptible bird (S) acquired bacteria indirectly from the environment at a rate β. The indirect transmission included the contamination of the environment ($\varphi(t)$), which was calculated from the shedding rate of bacteria (θ) from the infected birds (I) and the survival rate of the bacteria in the environment (ρ).

$$\phi(t) = e^{-\rho \quad t} \left(\phi(0) + \int_0^t e^{-\rho \quad \tau} \quad \theta I(\tau) \quad d\tau \right)$$

The transmission rate was reduced over time (ψ) to mimic age-related immunity development. An infected bird recovered at a rate γ and became susceptible again (Tables 2 and 3).

As for the SISIR model, the model distinguished infections per phylogenetic group. A susceptible bird (S_i) became infected indirectly via the environment with one of the three phylogenetic groups (i) at a rate β_i with contamination of the environment $\varphi(t)$ calculated as mentioned above. Then the infected bird (I_i) recovered at a rate γ_i and became susceptible again to another phylogenetic group. After two infections with different phylogenetic types, a bird recovered (R) and became immune to infection. Transmission and recovery rates were assumed to be specific to each phylogenetic group (Tables 2 and 3). In this model, the transmission rate was assumed to be time-independent and stable for the entire period.

We also explored the possibility of transmission happening either directly and indirectly or directly for the SIS model. As for the SISIR model, another model that incorporated only two phylogenetic groups was developed (Supplementally 1 and 2). All models were able to fit the observed data but based on the biological plausibility and to make a better comparison between two models, these were not used in the further discussion. Here, direct transmission occurred via contact between infected and susceptible animals and indirect transmission occurred via the pathogens in the environment excreted from infectious animals (Cortez and Weitz, 2013).

2.3. Parameterization of within-flock transmission dynamics

Within-flock transmission was parameterized using a longitudinal study conducted between June and November 2013 on a Dutch organic broiler farm by Huijbers et al. (2016). We used this data set because it was one of the few longitudinal studies, and we assume that it fits the current situation in which antibiotic use has reduced enormously (Nethmap-MARAN, 2021). Briefly, cloacal swabs were obtained from 100 broilers (80 tagged and 20 untagged) on days 1, 3, 4, 7, 10, 42, and 70 and analysed for the presence of ESBL-producing *E. coli*. The positive samples were further examined for phylogenetic group determination. The prevalence in tagged broilers was used in the SIS model. The prevalence in total broilers (tagged and untagged) was used for the SISIR model, because phylogenetic types were reported for both tagged and untagged animals.

Table 2
Transition equations on infectious states.

Transition	Description	Rate
SIS model		
S→I	Transition from susceptible (S) to infectious (I)	$-e^{-\psi t}\varphi(t)\beta S$
I → S	Transition from infectious to susceptible	$-\gamma I$
Ø → EE	Production of uncontaminated eggs (EE)	$bS + bI(1-l)$
Ø → EI	Production of contaminated eggs (EI)	bIl
EE → S	Hatching of susceptible chicks from uncontaminated eggs (EE)	δEE
EI→S	Hatching of susceptible chicks from contaminated eggs (EI)	$\delta(1-m)EI$
EI → I	Hatching of infectious chicks from contaminated eggs	δmEI
SISIR model		
$S \rightarrow I_i$	Transition from susceptible to infectious with phylogenetic group i	$-\varphi_i(t)\beta_i S$
$I_i \rightarrow S_{j,k}$ $I_i \rightarrow R$	Transition to susceptible for type j and k or recovery (R) from infectious with phylogenetic group i	$-\gamma_i I_i$
Ø → EE	Birth of uncontaminated eggs (EE)	$bS + bI_i(1-l)$
$Ø \rightarrow EI_i$	Birth of contaminated eggs (EI) with phylogenetic group i	bIl_i
EE → S	Hatching of susceptible chicks from uncontaminated eggs (EE)	δEE
$EI_i \rightarrow S$	Hatching of susceptible chicks from contaminated eggs (EI) with phylogenetic group i	$\delta(1-m)EI_i$
$EI_i \rightarrow I_i$	Hatching of infectious chicks from contaminated eggs with phylogenetic group i	δmEI_i

Ø: represents an empty set. β_i: Indirect transmission. i denotes phylogenetic type i in the SISIR model. ψ: transmission reduction rate. $\varphi_i(t)$: environmental contamination at time t. i denotes phylogenetic type i in the SISIR model. γ_i: Recovery rate. i denotes phylogenetic type i in the SISIR model. b: Laying rate. l: Pseudo vertical transmission rate. δ: Hatching rate. m: Hatching colonization rate.

Table 3
Prior distributions and posterior distributions (median with 95% credible interval) of transmission parameters used in SIS and SISIR model.

	Parameter description	Prior distribution	Posterior distribution (95% CI)
SIS model			
β(t)	Time-dependent indirect transmission rate	Uniform (0, 10)	4.20 (0.23, 9.51)
ψ	transmission reduction	Uniform (0, 1)	0.67 (0.28, 0.97)
γ	Recovery rate for the SIS model	Uniform (0, 1)	0.10 (0.02, 0.21)
Θ	Bacterial shedding rate	Uniform (0, 1)	0.55 (0.04, 0.96)
ρ	Bacteria survival rate in the environment	Uniform (0, 1)	0.51 (0.06, 0.97)
SISIR model			
β_1	Indirect transmission rate for phylogenetic type 1	Uniform (0, 10)	5.01 (0.57, 9.63)
β_2	Indirect transmission rate for phylogenetic type 2	Uniform (0, 10)	4.53 (0.65, 9.47)
β_3	Indirect transmission rate for phylogenetic type 3	Uniform (0, 10)	5.34 (0.94, 9.51)
γ_1	Recovery rate from phylogenetic type 1	Uniform (0, 1)	0.05 (0.03, 0.09)
γ_2	Recovery rate from phylogenetic type 2	Uniform (0, 1)	0.35 (0.052, 0.90)
γ_3	Recovery rate from phylogenetic type 3	Uniform (0, 1)	0.31 (0.02, 0.82)
Θ	Bacterial shedding rate	Uniform (0, 1)	0.52 (0.03, 0.97)
ρ	Bacteria survival rate in the environment	Uniform (0, 1)	0.77 (0.11, 0.99)

The parameters used in the within-flock models were fitted using the Approximate Bayesian Computation Sequential Monte Carlo (ABC-SMC) algorithm (Toni et al., 2009) implemented in the SimInf package (Widgren et al., 2019). Briefly, the ABC method consists of three steps. First, the parameters are sampled from prior distributions. Then, the generative model simulates a dataset using the parameters. Third, posterior distributions of the parameters are obtained by comparing the simulated data with the observed data and accepting proposed parameter values when the difference is within a pre-defined threshold. In our study, first, the models were run 100 times using random values from the prior parameter distributions, which were assumed to be uniform between 0 and 10 for transmission rate (β) or 0 and 1 for other parameters (ψ, γ, θ, ρ) (Table 3). The distance between the generated prevalence and the observed prevalence for each time point was obtained by the sum of squared differences of the prevalence, then the values were accepted at a rate of 0.9 per generation. The number of particles, the tolerance, the proportion of tolerance, and the generations was set to 500, 10,000, 0.9 and 100, respectively. The median values of the posterior distributions from 10 iterations were used to obtain the results for the basic scenario.

2.4. Initialization

Simulations were started by introducing around 2000 PS birds per age category with a 10% prevalence of ESBL-producing *E.coli* (Apostolakos et al., 2019; Dame-Korevaar et al., 2017). For the SISIR model, the prevalence of each phylogenetic group was assumed to be 92.3%, 7.7%, and 0.4%, respectively (Huijbers et al., 2016). The initial contamination of the environmental was assumed to be zero. The models were simulated for 4000 days with a burn-in period of 3000 days to eliminate the influence of these starting values.

2.5. Data analysis

The following outcomes of the SIS and SISIR model were used for analysis: the mean of the animal and flock level prevalence per production stage, the mean of the animal and flock level prevalence at slaughter, and human prevalence due to consumption of chicken meat. The animal level prevalence was derived by averaging the weighted average per model run over iterations. The flock level prevalence (the proportion of infected flocks) was calculated by dividing the number of flocks with at least one infected animal by the number of all flocks per run and averaged over iterations. The prevalence in broilers at slaughter time was obtained on day 42 of the production round. All results presented in this paper, unless otherwise stated, were analysed using data on simulation day 4000 from 10 iterations.

2.6. Evaluation of control measures

Currently, CE and probiotics, vaccination, and hygiene improvement are the major interventions that are considered to reduce the prevalence of *E. coli*. in practice (Becker et al., 2021; Swelum et al., 2021). The effectiveness of these control measures was evaluated by changing the values of the corresponding parameters. CE and vaccination were translated as a reduction in the shedding rate and the transmission rate, respectively. Improvement in hygiene was reflected by decreasing in the survival rate of bacteria in the environment. In short, four types of intervention scenarios were tested: (1) Reduction of shedding rate in PS birds and broilers, (2) Reduction of transmission rate in PS birds and broilers, (3) Decreasing the bacteria survival rate during the vacancy period in broiler farms, and (4) Decreasing the survival rate of the bacteria in PS and broiler farms. All reduction factors were determined based on literature as summarized in Table 4. Previous studies on CE reported a reduction of two to five log CFU/g bacteria in faeces and caecal content, thus the shedding rate was reduced to 1.0×10^{-2}, 1.0×10^{-3}, and 1.0×10^{-5} of the original value (Ceccarelli et al., 2017; Methner et al., 2019; Methner and Rösler, 2020; Nuotio et al., 2013). CE also reduced the transmission rate by 1.5 to 3-fold (Dame-Korevaar et al., 2020a), therefore, as a second intervention scenario, the transmission rate was reduced to 0.7 and 0.3 of the original value. Some studies reported that cleaning and disinfection almost eliminated the bacteria in the environment, thus the bacteria survival rate in the environment was reduced to 0.5, 0.25, 1.0×10^{-2}, and 0 of the original value (Gradel et al., 2004; K. Luyckx et al., 2015a; K. Y. Luyckx et al., 2015b). In addition, a 1.0×10^{-3} reduction in the transmission rate was also tested to compare with a 1.0×10^{-3} reduction in the shedding rate. Each scenario was run 10 times in both models and the mean prevalence

Table 4
Intervention scenarios and adjusted parameters and reduction values based on literature.

Parameters	Reduction factor*	Related Interventions (references)
Shedding rate (Parent Stock and broilers)	1.0×10^{-2} 1.0×10^{-3} 1.0×10^{-5}	CE treatment, vaccination (Ceccarelli et al., 2017;Methner et al., 2020;Methner et al., 2019;Nuotio et al., 2013)
Transmission rate (Parent Stock and broilers)	0.7 0.3 1.0×10^{-3}	CE treatment, vaccination, improvement of hygiene (indirect transmission) (Dame-Korevaar et al., 2020)
Bacteria survival rate during the vacancy period (Broilers)	0.5 0.25 1.0×10^{-2} 0	Cleaning and disinfection (Gradel et al., 2004; Hao et al., 2013; K. Luyckx et al., 2015a; K. Y. Luyckx et al., 2015b)
Bacteria survival rate (Parent Stock and broilers)	1.0×10^{-2} 0	Values based on cleaning and disinfection (Gradel et al., 2004; Hao et al., 2013; K. Luyckx et al., 2015a; K. Y. Luyckx et al., 2015b)

* Reduction factor shows the reduction from the original value (e.g., reduction of 1.0×10^{-2} means the value used was the 1.0×10^{-2} of the original parameter value see Table 3).

at slaughter in broiler farms was compared to that of the basic scenario.

2.7. Quantitative Microbiological Risk Assessment

Simulated prevalence at slaughter in broiler farms was used to calculate human prevalence due to consumption of chicken meat (Table 5, Fig. 3).

First, to calculate the prevalence at consumption level (*A*), animal level prevalence per simulated flock was multiplied by the reduction of prevalence from raw chicken meat to chicken meat at the moment of consumption (0.102). Then (*A*) was multiplied with the size of the flock and the probability of becoming an ESBL-producing *E. coli* carrier after consuming a contaminated portion of chicken ($P_{carriership}$; 1.19×10^{-3}), and the result was summed over all simulated flocks. The probability ($P_{carriership}$) was estimated by literature research assuming a Beta Poisson dose-carriership relation (E. Evers, personal communication):

$P_{carriership} = 1 - \left(1 + \frac{D}{\omega}\right)^{-\alpha}$ Where D is the dose of *E. coli* ingested (number of CFU), estimated at 1.75 CFU (Evers et al., 2017) and α and ω are parameters, estimated at 0.248 and 365.

The summed result was then multiplied with the number of chicken portions consumed per year by the population in the Netherlands and divided by the total simulated number of broilers, to obtain the yearly incidence of ESBL-producing *E. coli* in humans in the Netherlands (*B*). Finally, the prevalence of ESBL-producing *E. coli* in humans was obtained by multiplying (*B*) with the mean duration of ESBL-producing *E. coli* carriership and dividing by the human population size of the Netherlands (Fig. 3).

2.8. Sensitivity analysis

Sensitivity analysis was performed to assess the impact of variation in parameters on the outcome. All parameters in the models were examined except for those determined the demography. Using Latin Hypercube Sampling (LHS), ABC-fitted parameters were sampled from the 2.5% and 97.5% percentiles of the posterior distribution (Table 3). For pseudo vertical transmission and hatching colonization rates, the 95% confidence intervals from the literature values were used, which were 0.0036 (95% CI: 5.81×10^{-6}, 0.014) and 0.011 (95% CI: 0.0018, 0.025) (Projahn et al., 2017) (Table 1). The sample size was set to 10 times the number of parameters used in the corresponding model, which was 70 and 100 for the SIS and SISIR model, respectively. The linear regression coefficients were obtained between the sampled parameters and the following three outputs on simulation day 4000: (1) the average animal level prevalence for each production stage (the sum of the animal level prevalence in all flocks divided by the number of flocks), (2) the flock level prevalence for each production stage (the proportion of infected flocks), and (3) the mean animal level prevalence at slaughter (the sum of the animal level prevalence divided by the number of broiler farms on day 42 in a production cycle). Broiler farms that are in the vacancy period were excluded from the outcomes (1) and (2).

Table 5
Factors for the Quantitative Microbiological Risk Assessment.

Factor	Value	Reference
Reduction of prevalence from raw chicken meat to chicken meat at the moment of consumption	0.102	Evers et al. (2017)
Probability of becoming an ESBL-producing *E. coli* carrier after consuming a contaminated chicken portion	1.19×10^{-3}	Evers et al. (2017); E. Evers, personal communication
Number of chicken portions consumed per year by the human population in the Netherlands	1.75×10^{9}	Evers et al. (2017)
Mean duration of ESBL-producing *E. coli* human carriership	1.1 years	Teunis et al. (2018)
Human population size in the Netherlands	1.741×10^{7}	CBS Statline 2022b

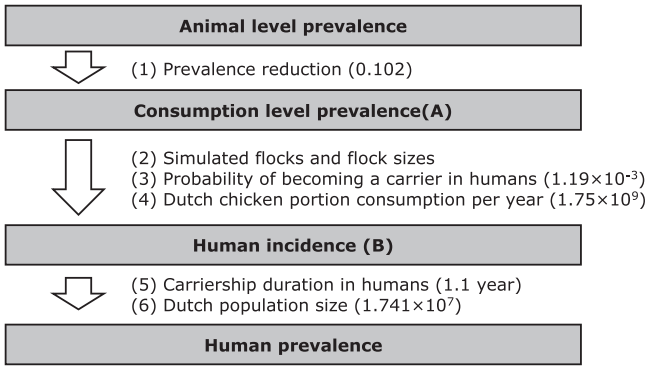


Fig. 3. Assessment of human prevalence due to consumption of chicken meat.

2.9. Simulation method

The transmission and ABC simulations were performed using the SimInf package (version 8.2.0.9000) in R (version 4.0.4). SimInf is a modelling framework for data-driven modelling and simulation of stochastic disease spread within and among subpopulations (Widgren et al., 2019).

3. Results

3.1. Parameterization of within-flock transmission dynamics

The ABC-fitted posterior parameter distributions are given in Table 3. Both SIS and SISIR model were able to capture the fluctuation in prevalence that was observed in the study (Fig. 4). In the SISIR model, the estimated transmission rates per phylogenetic group were in the same range, but the infection duration varied among the phylogenetic types.

3.2. Transmission between the production stages and estimated human prevalence

Overall, the animal level prevalence started at around 7% in PS, decreased to 0.02 to 0.03% in hatcheries, and increased in broilers to 36 to 54% (Table 6). Human prevalence due to consumption of contaminated chicken meat was calculated at around 0.12% and 0.15%. The contribution of chicken meat consumption to human ESBL carriage can then be estimated at a proportion of 0.014 to 0.030.

The variability between flocks and simulation runs are expressed as the standard deviation (SD) and the 95% simulation intervals (Table 6). The SD shows the variation between flocks within a simulation, representing the variation in the infection states between the flocks. The 95% intervals of the mean prevalence and the SD shows the variation between the model iterations. For example, the mean animal prevalence in the broiler farms in the SIS model was 53.75% (53.32 - 55.06) with a SD of 38.92 (38.56 - 39.28). This means the average animal prevalence over 10 iterations was 53.75% with a variation of 38.92% between flocks. The reason for the large SD, unlike other production stages, is because the flocks followed different production cycles (e.g., one flock is on day 1, when almost all birds are susceptible while the other flock is on day 4 when almost all birds are infected). The credibility of our models is supported by the narrow range of the 95% simulation intervals for both the mean animal prevalence and SD, indicating a small variation among the simulations. The flock prevalence was 86.58% (84.88 - 88.64) with a SD of 1.39% over the iterations. The human prevalence by consuming meat from these broilers was calculated at 0.12% (0.09 - 0.14) with a SD of 0.01% over the iterations.

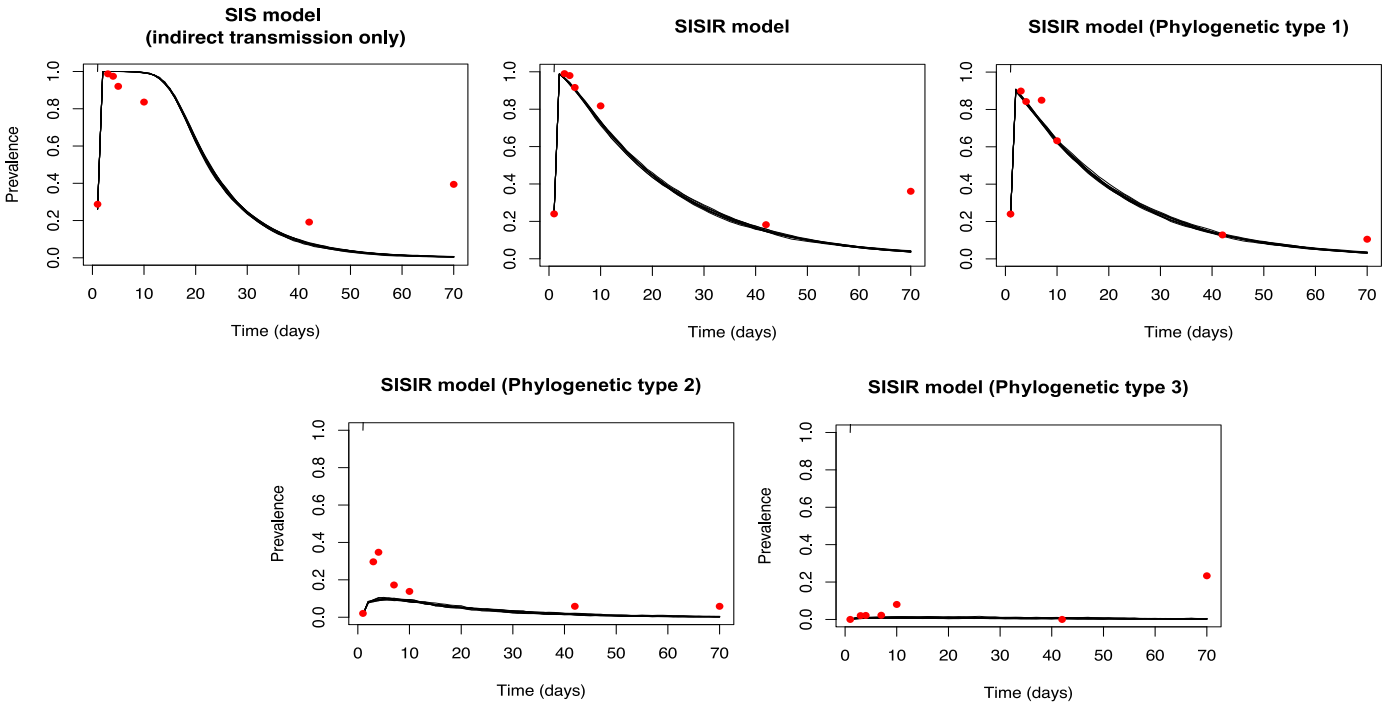


Fig. 4. ABC-simulated prevalence (black line) and observed data (red points). Top left: SIS model. Top middle: SISIR model. Top right and bottom: Specific phylogenetic groups in the SISIR model.

Table 6
The mean and standard deviation of simulated flock and animal level prevalence at all production stages and human prevalence due to consumption.

Stage	SIS model		SISIR model	
	Mean (95% Interval)	SD (95% Interval)	Mean (95% Interval)	SD (95% Interval)
Parent Stock	7.30 (7.29, 7.31) 100.00 (100.00, 100.00)	7.74 (7.73, 7.75) 0.00	6.82 (6.81, 6.84) 100.00 (100.00, 100.00)	5.80 (5.79, 5.82) 0.00
Hatcheries	0.03 (0.03, 0.03) 100.00 (100.00, 100.00)	0.01 (0.01, 0.01) 0.00	0.02 (0.02, 0.03) 100.00 (100.00, 100.00)	0.01 (0.01, 0.01) 0.00
Broilers	53.75 (53.32, 55.06) 86.58 (84.88, 88.64)	38.92 (38.56, 39.28) 1.39	36.34 (35.72, 37.06) 86.04 (84.88, 88.05)	27.50 (27.19, 27.95) 1.11
Slaughter	8.98 (7.08, 10.10) 90.00 (70.96, 100.00)	2.34 (0.28, 4.70) 10.91	11.47 (9.88, 12.89) 84.62 (76.92, 92.31)	5.03 (3.81, 5.92) 6.28
Human	0.12 (0.09, 0.14)	0.01	0.15 (0.13, 0.17)	0.01

Top row: Mean animal-level prevalence in % (95% simulation interval) and SD (95% simulation interval). Bottom row: flock-level prevalence in % (95% simulation interval) and SD. The SD shows the variation between flocks within the simulation. The 95% simulation intervals of the mean and the SD shows the variation between iterations.

3.3. Intervention scenarios

The effect of the intervention scenarios on the animal level prevalence at slaughter and transmission to humans differed between the models (Fig. 5). In the SIS model, both broiler and human prevalence were reduced by the four types of interventions: (1) reduction of the shedding rate, (2) reduction of the transmission rate, (3) reduction of the bacteria survival rate during the vacancy period in broiler farms, and (4) reduction of the bacteria survival rate in PS and broiler farms. To reduce the prevalence, a reduction of more than 1.0×10^{-2} and 0.3 was needed for the shedding and transmission rate, respectively. The impact of the reduction of the shedding and the transmission rate on the outcome was compared by reducing both rates to the 1.0×10^{-3} of the original value, resulting in a similar negative impact on the prevalence.

As for the SISIR model, only interventions (3) and (4) were able to decrease the prevalence in animals and humans. In both settings, the prevalence decreased when the survival rate of the bacteria was reduced to more than 1.0×10^{-2} of the original value. On the other hand, the prevalence increased unexpectedly when the shedding rate or the transmission rate was reduced to 1.0×10^{-5} or 1.0×10^{-3} of the original value. This was because the reduction of the shedding and transmission rates slowed the transmission between the birds, resulting in fewer recovered birds after two infections compared to the basic scenario; at slaughter, there were 665 and 49 more birds in the first infection and second infection, respectively, and 948 fewer recovered birds per farm when the shedding rate was reduced to 1.0×10^{-5} of the original value.

3.4. Sensitivity analysis

Table 7 shows the results of the regression coefficient analysis for both models ($p < 0.05$). For the SIS model, only the recovery rate (γ) had a negative impact on the flock level prevalence in PS farms. In hatcheries, only pseudo vertical transmission rate (l) positively affected the flock level prevalence. In broiler farms, γ and the transmission reduction (ψ) negatively correlated with the animal level prevalence and positively with l. Similarly, at the flock level, the prevalence was strongly negatively correlated with γ and positively with l. The animal level prevalence at slaughter was strongly negatively correlated with γ and ψ. For the SISIR model, only the recovery rate for phylogenetic group 1 (γ_1) had a negative impact on the animal level prevalence in PS farms. In hatcheries, l positively affected the flock level prevalence. In broiler farms, the animal level and farm prevalence and the prevalence at

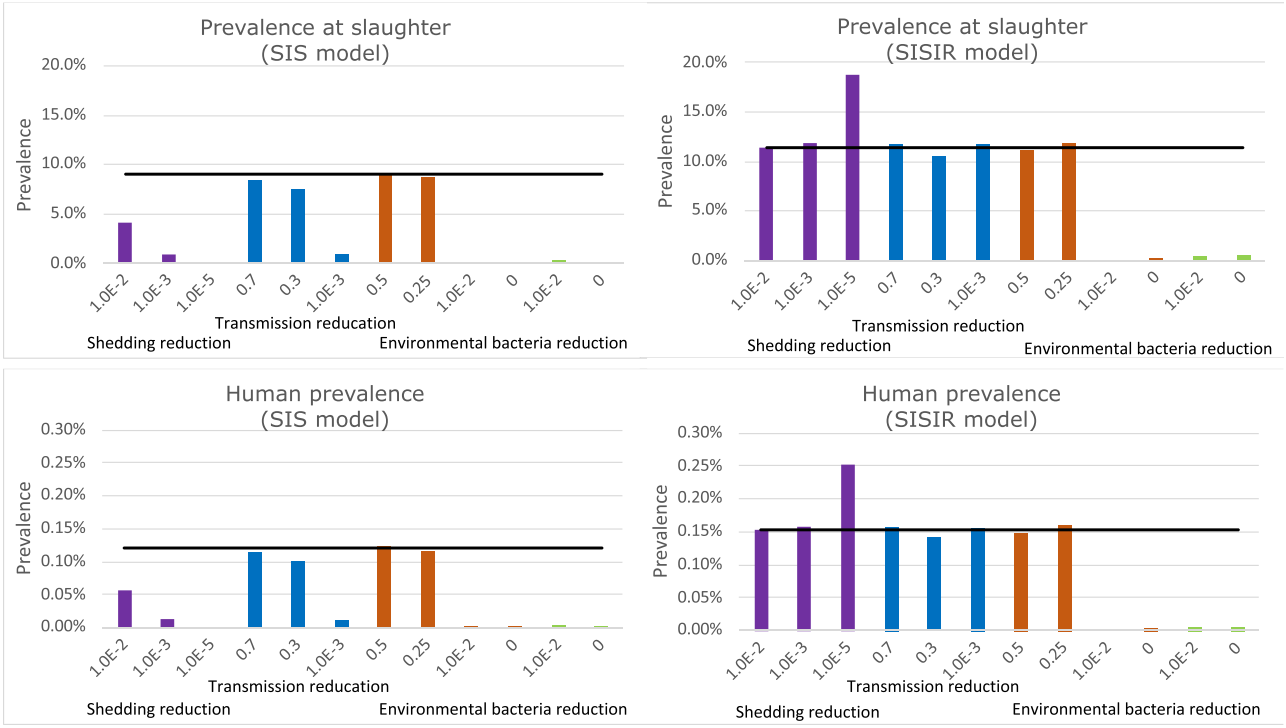


Fig. 5. Effect of interventions on the simulated animal-level prevalence at slaughter (Top) and human prevalence (Bottom) for the SIS model (left) and SISIR model (right). X axis: reduction of parameters. Horizontal black line: the prevalence from the base scenario. Purple bar: effect of reduction in the shedding rate (intervention (1)). Blue bar: effect of reduction in the transmission rate (intervention (2)). Orange bar: effect of reduction in the bacteria survival rate during vacancy period (intervention (3)). Green bar: effect of reduction in the bacteria survival rate (intervention (4)).

Table 7
Regression coefficients of the model parameters on the outcomes.

		β	γ			Θ	ρ	ψ	l	m
SIS model										
(1)	PS	0.0	-0.4			0.0	0.0	-0.1	-	-
	Hat	0.0	-0.0			0.0	0.0	0.0	0.1	-
	Bro	0.0	**-2.5**				0.4	**-0.5**	**15.4**	-
(2)	PS	-	**-0.7**			-	-	-0.1	-	-
	Hat	-	-			-	-	-	**1.7**	-
	Bro	-	**-1.7**			-	0.4	-	**23.7**	-
(3)	Slaugh	0.0	**-2.4**			-	0.2	**-0.5**	-	-
SISIR model		**β_1**	**γ_1**	**γ_2**	**γ_3**	**Θ**	**ρ**		**l**	**m**
(1)	PS	0.0	**-1.0**	-	-0.0	-	-		-	-
	Hat	-	-0.0	-	-0.0	-	-		0.1	-
	Bro	-	**-0.5**	-	-	-	-		**3.2**	**1.5**
(2)	PS	-	-	-	-	-	-		-	-
	Hat	-	-	-	-	-	-		**1.4**	-
	Bro	-	**-0.7**		-0.0	-			**8.3**	**4.2**
(3)	Slaugh	-	**-0.3**		-	-0.0	-		**1.2**	**0.4**

(1) the animal prevalence per production stage, (2) the proportion of infected flocks per production stage, and (3) the prevalence at slaughter. Coefficients less than − 0.5 or more than 0.5 are in bold. ($p < 0.05$) PS: Parent Stock. Hat: Hatcheries. Bro: Broiler. Slaugh: Broilers at slaughter β_i: Indirect transmission rate. *i* denotes phylogenetic type *i* in the SISIR model. γ_i: Recovery rate. *i* denotes phylogenetic type *i* for the SISIR model. θ: Bacterial shedding rate. ρ: Bacteria survival rate in the environment. ψ: transmission reduction rate. l: Pseudo vertical transmission rate. m: Hatching colonization rate.

slaughter was strongly negatively correlated with γ_1 and strongly positively correlated with *l* and the hatching colonization rate (*m*). Here a strong correlation is used when the coefficient was less than − 0.5 or more than 0.5.

4. Discussion

The models in this study captured the transmission dynamics of ESBL-producing *E. coli* by including various aspects of the infection characteristics as well as the features of the Dutch broiler production chain and evaluated the effects of intervention scenarios. To our knowledge, this is the first study that mechanistically modelled the reported decrease in the ESBL-producing *E. coli* prevalence during a production round (Apostolakos et al., 2019; Dame-Korevaar et al., 2017; Dierikx et al., 2013; Huijbers et al., 2016; Laube et al., 2013). Two models were developed based on two different assumptions on the prevalence reduction mechanism: time-dependent transmission reduction and partial immunity to phylogenetic groups. Some studies reported that the community richness of the microbiota in chickens increases as they age (Ballou et al., 2016; Jurburg et al., 2019; Lu et al., 2003) and that the development of the microbiota was associated with immune cell activation and nutrition intake (Meijerink et al., 2020; Oakley et al.,

2014). Furthermore, antibiotic resistance levels in intestinal *E. coli* decreased as broilers become older (Diarra et al., 2007). We assumed that age-related change in the microbiota influenced the susceptibility of chickens and modelled it as a time-dependent reduction in transmission (ψ) using the SIS model. The time-dependent reduction was estimated at 0.67, which implies the transmission is reduced exponentially by 0.67 per day. More longitudinal studies that focus on the shifts in susceptibility and immunity of chicks are needed to validate this estimated value. Another assumption on the underlying mechanism of the prevalence reduction was made based on the susceptibility differences and partial immunity against phylogenetic groups. As repeated shifts in the phylogenetic group are believed to be the main cause of the persistence of the bacteria in poultry farms (Apostolakos et al., 2019), we used the SISIR model to demonstrate the transmission. To support this assumption, longitudinal research that focusses on the dynamics of phylogenetic groups, including rates of transmission, duration of the infection, and the changes in susceptibility of chickens in all production stages, is needed. Furthermore, an experimental longitudinal study on parent stock birds reported that the type of plasmids might influence the ability of the conjugation process, thus leading to the decline in the prevalence (Dame-Korevaar et al., 2017). More focus might be also needed not only on phylogenetic types but also on the plasmid level.

According to our models, the infectious period was overall shorter and the transmission rate was higher than in the previous study (Huijbers et al., 2016) which was driven by the initial steep increase in the prevalence (Table 3). At slaughter (day 42), the animal level prevalence from both models was lower than 19.1% from the study by Huijbers et al. (2016) but more or less consistent with the reported value of 9.8% in Dutch broilers (Nethmap-MARAN, 2021). Although the reported prevalence might be not comparable because the current model was built on data from an organic farm, thus underestimating the simulated prevalences because of the differences in farm management, the models were still able to capture the transmission dynamics in the broiler production chain. One of the strong points of using data from an organic farm is that it has a long rearing period compared to other conventional farms, which implies that more data points available. Considering the recent trend of reduction in antimicrobial use (Nethmap-MARAN, 2021) an increased rearing period in conventional farms might occur, making the model applicable not only to organic farms but also to conventional farms. Furthermore, the number of organic farms is expected to increase in accordance with the current European policy, including the Regulation (EU) 2019/6 and the Farm to Fork strategy, which increases the relevance of our models (European Commission, 2018, 2019).

The simulated animal level prevalence in broilers was higher than that of the PS birds. This can be explained by the duration of the production cycle; birds in the PS farms were older than the broilers which implied that the transmission rate was lower (SIS model), or the birds were already recovered from two infections (SISIR model). The shifts in animal level prevalence through the production chain were similar to the pattern reported in the modelling study by Plaza-Rodriguez et al. (2018). The prevalence started at around 10% in PS farms, dropped in hatcheries, and then increased to the highest in broiler farms. The prevalence of ESBL/AmpC-producing *E. coli* in PS farms in Italy was reported at 92.5% in 1-day-old and 20% in 30-week-old birds (Apostolakos et al., 2019). In Finland, it was reported to be 26.7% in 46-week--old birds. These values are not comparable because the PS farms in our models consisted of different age groups, and further observational study is needed to better understand the transmission dynamics in PS farms. At the hatchery level, the estimated level of egg contamination was within the range of the reported values, which ranged from 0% (Oikarainen et al., 2019) to 3.8% (Mezhoud et al., 2016).

Contamination in the hatchery is an important source of resistant bacteria in broiler flocks (Heinemann et al., 2020). The actual pseudo vertical transmission from contaminated eggs to chicks is low for ESBL/AmpC with only 1 colonized hatchling out 280 contaminated eggs (Projahn et al., 2017). Our simulations do, however, show that even a small proportion of contaminated hatchlings can result in major outbreaks of ESBL producing E. coli in a broiler farm. We simplified the dynamics in the hatchery by disregarding the environmental contamination assuming daily cleaning and disinfection and no mixing of batches of hatchlings. This is of course idealized and hatchery environments might be contaminated. We did, however, not consider interventions at hatcheries and therefore this will not alter our conclusions on the most effective interventions. The early life stages are an point in attention. The effect of contaminated hatcheries would be an increase of contaminated hatchlings which is also achieved by assuming higher pseudo-vertical transmission. Our sensitivity analysis shows that this is an important factor for the number of contaminated flocks and overall prevalence in flocks.

Control measures that can reduce the shedding and transmission rates may be only effective when the transmission dynamics follow the SIS model. In this model, exponential transmission rate reduction resulted in a faster reduction in the prevalence, while in the SISIR model, the transmissibility was assumed to be constant, and the reduction levels used in the intervention scenarios were not enough to influence the prevalence. In practice, vaccination or CE can reduce the shedding of bacteria which can then decrease transmission between birds. Even though the effect level is unknown, this can be further explored by combining the first and second scenarios. Administration of CE or vaccination should be done in the early stage of life, as birds started excreting the bacteria within 24 h after inoculation (Ceccarelli et al., 2017). However, when the shedding rate of the SISIR model was reduced to 1.0×10^{-5}, the prevalence at slaughter increased unexpectedly. This implies that interventions that slow the spread of the bacteria can increase the prevalence because a bird needs longer time to become infected and to reach the recovered status. If the transmission dynamics follow the SISIR model, such intervention should not be recommended.

For both models, the most effective control measure can be farm management that aims to reduce the number of bacteria in the environment. Between production rounds such measures include but are not limited to washing the premises with water, detergents, and disinfectants between production rounds (Gradel et al., 2004; Hao et al., 2013; K. Luyckx et al., 2015a; K.Y. Luyckx et al., 2015b). We did, however, also show that reducing survival in the environment with at least 99% during production rounds is effective. Cleaning and disinfection is practically impossible during production rounds, and to our knowledge no measures that do decrease survival in the environment are known. Therefore this option is currently purely hypothetical, although our outcomes do justify investigations in to new and innovative approaches, such as application of competitive exclusion products in the environment or special bedding. Furthermore, the control measure should be applied in combination with biosecurity measures to prevent transmission between stages, as a study suggested that half of the genotypes were originated from the previous stage (Apostolakos et al., 2019). On farm hatching could reduce the initial contamination by eliminating cross contamination in the hatchery. A future addition to our model could be the inclusion of on farm hatching.

Furthermore, we need to explore cost-effective methods that can be universally applicable to every production stage. The alternative but costly option can be routinely collecting and checking the environmental samples. More quantitative studies on the cost effectiveness of such management and the influence on the public health are expected.

According to the SIS and SISIR model, the ESBL-producing *E. coli* prevalence in humans due to chicken consumption was only 0.12% and 0.15%, respectively (Table 6). Considering the prevalence in the general Dutch population, which was reported to be around 5.0% to 8.6% (Reuland et al., 2016; van den Bunt et al., 2019), chicken meat consumption can still be considered a minor contribution to human exposure at a proportion of around 0.014 to 0.030. Our estimate is similar to the epidemiological study that estimated that chicken meat accounted for a proportion of 0.045 of intestinal carriage of ESBL or pAmpC gene in

the general population (Mughini-Gras et al., 2019). We used the animal level prevalence at slaughter as calculated by the models as the fresh chicken meat prevalence which was 8.98% and 11.47% for the SIS and the SISIR model, respectively (Table 6). These values were much lower than the reported value of 67.0% (Evers et al., 2017) and thus might have underestimated the risk. However, considering the recent downward trend in prevalence in broilers, the values used in this study can be regarded as relevant enough. We ignored the effect on prevalence from the slaughter process because even though the process reduced bacteria concentration, it seemed to have little effect on prevalence (Pacholewicz et al., 2015). As a previous study revealed that the gene distribution in chicken meat at retail was distant from that of broilers and chicken meat at the slaughterhouse (Dorado-García et al., 2018), an investigation on cross-contamination is needed to better understand the possible source of human exposure.

In the SIS model, the animal level prevalence within broiler flocks was sensitive to parameters that determined the duration of infection (γ) and the reduction of transmission (ψ). In contrast, the flock level prevalence was not influenced by ψ, because outbreaks can still occur through the environmental contamination from previous production rounds, or from the influx from the hatcheries, such as by pseudo vertical transmission (l), which had a positive correlation. The flock level prevalence was sensitive to γ and l, indicating that reducing these factors is important for controlling the bacteria at a national level. In the SISIR model, both animal and flock level prevalence in broiler farms were sensitive to the parameters that determined the duration of infection (γ_1), pseudo vertical transmission (l), and colonization at hatching (m). Both models indicated that controlling the duration of infection of broilers as well as the contamination level of eggs are the main options to be explored to reduce the spread of bacteria.

There are some limitations in the modelling. Our model assumed that one farm consisted of one flock and did not include transmission between flocks within the same farm or the effect of spatial separation on between-farm transmission, both of which were identified as transmission routes in a previous study (Dame-Korevaar et al., 2019). There are several limitations specific to the SISIR model. It is highly unlikely that an animal would get immunity after two infections. Considering the infection duration and the variance in phylogenetic groups, at least three or four infections should occur during the production cycle in broilers and even more for PS birds. The major phylogenetic groups found in the study by Huijbers et al. (2016) were A and B1 as was also the case in other reports (Evers et al., 2021; Zurfluh et al., 2014). We assumed that the constitution of the phylogenetic groups was the same throughout the production chain, although a study has pointed out that it can differ among the production stages (Apostolakos et al., 2019). To improve the model, it might be an option to incorporate more phylogenetic groups or add a time-related immunity decrease, but at the same time it will make the modelling more complex, resulting in the increased assumptions due to data unavailability and difficulties in translating the results (Katsma et al., 2007).

The availability of the data on vertical transmission should also be addressed in future studies. Projahn et al. (2017) reported that the outer surface of one out of 280 eggs (0.36%) was ESBL-/pAmpC-producing enterobacteria positive after disinfection, thus the pseudo vertical transmission rate (l) was set at 0.0036. The same study reported that three out of 280 recently hatched chicks were already colonized with enterobacteria (Projahn et al., 2017). Although none of them were positive for ESBL-/pAmpC-producing enterobacteria, this value was used as the hatching colonization rate (m) in our study for worst case scenario. The sensitivity analysis shows that the outcomes for broiler farms is sensitive to these parameters. Although the studies by Projahn et al. (2017) and Oikarainen et al. (2019) could not determine pseudo vertical transmission, other studies indicated the possible transmission from hatcheries to farms as reviewed by Dame-Korevaar et al. (2019).

5. Conclusions

Regardless of the transmission mechanisms used in the current investigation, the results suggest that improving farm management to eliminate the bacteria from the environment will be the most effective intervention to reduce the health risk, both for animals and humans, of ESBL-producing *E. coli* in the broiler production chain. Contribution of chicken meat to ESBL-producing *E. coli* prevalence in humans is low. However, it is still important to monitor ESBL-producing *E. coli* and try to reduce them in the broiler production chain as much as possible because they can serve as the source of antimicrobial resistance genes.

Funding

This work within the One Health EJP Full-Force project was supported by funding from the European Union's Horizon 2020 Research and Innovation programme: One Health European Joint Programme [grant number 773830].

CRediT authorship contribution statement

Furusawa Minori: Conceptualization, Formal analysis, Investigation, Methodology, Software, Writing – original draft, Writing – review & editing. **Fischer Egil A.J.:** Conceptualization, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing. **Evers Eric G.:** Conceptualization, Methodology, Supervision, Writing – review & editing. **Widgren Stefan:** Formal analysis, Methodology, Software, Writing – review & editing.

Declaration of Competing Interest

none.

Acknowledgements

We would like to thank the colleagues of the infectious disease modelling group at the division of Farm Animal Health, Department Population Health Sciences Faculty of Veterinary Medicine for their helpful feedback. This work was based on previous work by Alana Dekker (MSc thesis Epidemiology).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.prevetmed.2024.106121.

References

Apostolakos, I., Mughini-Gras, L., Fasolato, L., Piccirillo, A., 2019. Assessing the occurrence and transfer dynamics of ESBL/PAmpC-producing *Escherichia coli* across the broiler production pyramid. PloS One 14 (5), e0217174.

Archer, G.S., Lee Cartwright, A., 2017. Incubating and Hatching Eggs. https://alec.unl.edu/documents/cde/2017/livestock-management/incubating-and-hatching-eggs-2017.pdf (accessed 16 October 2022).

Ballou, A.L., Ali, R.A., Mendoza, M.A., Ellis, J.C., Hassan, H.M., Croom, W.J., Koci, M.D., 2016. Development of the chick microbiome: how early exposure influences future microbial diversity. Front. Vet. Sci. 3 (January), 2.

Becker, E., Projahn, M., Burow, E., Käsbohrer, A., 2021. Are there effective intervention measures in broiler production against the ESBL/AmpC producer *Escherichia coli*? Pathog 10 (5). https://doi.org/10.3390/pathogens10050608.

Ceccarelli, D., van Essen-Zandbergen, A., Smid, B., Veldman, K.T., Boender, G.J., Fischer, E.A.J., Mevius, D.J., van der Goot, J.A., 2017. Competitive exclusion reduces transmission and excretion of extended-spectrum-β-lactamase-producing *Escherichia coli* in broilers. Appl. Environ. Microbiol. 83 (11) https://doi.org/10.1128/AEM.03439-16.

Chong, Y., Ito, Y., Kamimura, T., 2011. Genetic evolution and clinical impact in extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*. Infect. Genet. Evol. 11 (7), 1499–1504.

Cortez, M.H., Weitz, J.S., 2013. Distinguishing between indirect and direct modes of transmission using epidemiological time series. Am. Nat. 181 (2), E43–E54.

Dame-Korevaar, A., 2020. Interventions to reduce the spread of ESBL/PAmpC-Producing *E. coli* in the broiler production pyramid. Utrecht Univ.

Dame-Korevaar, A., Fischer, E.A.J., Stegeman, A., Mevius, D., van Essen-Zandbergen, A., Velkers, F., van der Goot, J., 2017. Dynamics of CMY-2 producing *E. coli* in a broiler parent flock. Vet. Microbiol. 203 (May), 211–214.

Dame-Korevaar, A., Fischer, E.A.J., van der Goot, J., Stegeman, A., Mevius, D., 2019. Transmission routes of ESBL/PAmpC producing bacteria in the broiler production pyramid, a literature review. Prev. Vet. Med. 162 (January), 136–150.

Dame-Korevaar, A., Fischer, E.A.J., van der Goot, J., Velkers, F., Ceccarelli, D., Mevius, D., Stegeman, A., 2020a. Early life supply of competitive exclusion products reduces colonization of extended spectrum beta-lactamase-producing *Escherichia coli* in Broilers. Poult. Sci. 99 (8), 4052–4064.

Dame-Korevaar, A., Kers, J.G., van der Goot, J., Velkers, F.C., Ceccarelli, D., Mevius, D.J., Stegeman, A., Fischer, E.A.J., 2020b. Competitive exclusion prevents colonization and compartmentalization reduces transmission of ESBL-producing *Escherichia coli* in Broilers. Front. Microbiol. 11 (November), 566619.

Dekker, A. 2019. The Importance of Pseudo-Vertical and Horizontal Transmission in the Spread of Extended-Spectrum Beta- Lactamase Producing Bacteria: A Modelling Study. Utrecht Univ.

van den Bunt, G., van Pelt, W., Hidalgo, L., Scharringa, J., de Greeff, S.C., Schürch, A.C., Mughini-Gras, L., Bonten, M.J.M., Fluit, A.C., 2019. Prevalence, risk factors and genetic characterisation of extended-spectrum beta-lactamase and carbapenemase-producing Enterobacteriaceae (ESBL-E and CPE): a community-based cross-sectional study, the Netherlands, 2014 to 2016. Eur. Surveill. 24 (41) https://doi.org/10.2807/1560-7917.ES.2019.24.41.1800594.

Diarra, M.S., Silversides, F.G., Diarrassouba, F., Pritchard, J., Masson, L., Brousseau, R., Bonnet, C., Delaquis, P., Bach, S., Skura, B.J., Topp, E., 2007. Impact of feed supplementation with antimicrobial agents on growth performance of broiler chickens, *Clostridium perfringens* and *Enterococcus* counts, and antibiotic resistance phenotypes and distribution of antimicrobial resistance determinants in *Escherichia coli* Isolates. Appl. Environ. Microbiol. 73 (20), 6566–6576.

Dierikx, C.M., van der Goot, J.A., Smith, H.E., Kant, A., Mevius, D.J., 2013. Presence of ESBL/AmpC-producing *Escherichia coli* in the broiler production pyramid: a descriptive study. PloS One 8 (11), e79005.

Dorado-García, A., Smid, J.H., van Pelt, W., Bonten, M.J.M., Fluit, A.C., van den Bunt, G., Wagenaar, J.A., Hordijk, J., Dierikx, C.M., Veldman, K.T., de Koeijer, A., Dohmen, W., Schmitt, H., Liakopoulos, A., Pacholewicz, E., Lam, T.J.G.M., Velthuis, A.G., Heuvelink, A., Gonggrijp, M.A., Heederik, D.J.J., 2018. Molecular Relatedness of ESBL/AmpC-Producing *Escherichia coli* from humans, animals, food and the environment: a pooled analysis. *J. Antimicrob. Chemother.* 73 (2), 339–347.

EFSA, ECDC, 2022. The European Union Summary Report on Antimicrobial Resistance in zoonotic and indicator bacteria from humans, animals and food in 2019–2020. EFSA J. 20 (3) https://doi.org/10.2903/j.efsa.2022.7209.

EFSA Panel on Animal Health and Welfare, 2010. Scientific opinion on welfare aspects of the management and housing of the grand-parent and parent stocks raised and kept for breeding purposes. EFSA J. 8 (7), 1667.

Ellen, H.H., F.R. Leenstra, R.A. van Emous, C.M. Groenestein, J. van Harn, Plm van Horne, Young, I.C. the, M. Kense, D.J. Mevius, and J.A. Wagenaar, 2012. Vleeskuikenproductiesystemen in Nederland. Wageningen UR Livestock Research. https://edepot.wur.nl/222179.

European Commission, 2018. Regulation (EU) 2019/6 of the European Parliament and of the Council of 11 December 2018 on Veterinary Medicinal Products and Repealing Directive 2001/82/EC. December 1, 2018. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32019R0006&qid=1619008214855 (accessed 14 April 2021).

European Commission, 2019. COMMUNICATION FROM THE COMMISSION TO THE EUROPEAN PARLIAMENT, THE EUROPEAN COUNCIL, THE COUNCIL, THE EUROPEAN ECONOMIC AND SOCIAL COMMITTEE AND THE COMMITTEE OF THE REGIONS The European Green Deal. December 11, 2019. https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:52019DC0640&from=EN (accessed 14 April 2021).

Evers, E.G., Pielaat, A., Smid, J.H., van Duijkeren, E., Vennemann, F.B.C., Wijnands, L. M., Chardon, J.E., 2017. Comparative exposure assessment of ESBL-producing *Escherichia coli* through meat consumption. PloS One 12 (1), e0169589.

Gradel, K.O., Jørgensen, J.C., Andersen, J.S., Corry, J.E.L., 2004. Monitoring the efficacy of steam and formaldehyde treatment of naturally salmonella-infected layer houses. J. Appl. Microbiol. 96 (3), 613–622.

Hao, X.X., Li, B.M., Wang, C.Y., Zhang, Q., Cao, W., 2013. Application of slightly acidic electrolyzed water for inactivating microbes in a layer breeding house. Poult. Sci. 92 (10), 2560–2566.

Heinemann, C., Leubner, C.D., Savin, M., Sib, E., Schmithausenm, R.M., Steinhoff-Wagner, J., 2020. Research note: tracing pathways of entry and persistence of facultative pathogenic and antibiotic-resistant bacteria in a commercial broiler farm with substantial health problems. Poult. Sci. 99 (11), 5481–5486. https://doi.org/10.1016/j.psj.2020.08.050.

Huijbers, P.M.C., Graat, E.A.M., van Hoek, A.H.A.M., Veenman, C., de Jong, M.C.M., van Duijkeren, E., 2016. Transmission dynamics of extended-spectrum β-lactamase and AmpC β-lactamase-producing *Escherichia coli* in a broiler flock without antibiotic use. Prev. Vet. Med. 131 (September), 12–19.

Jurburg, S.D., Brouwer, M.S.M., Ceccarelli, D., van der Goot, J., Jansman, A.J.M., Bossers, A., 2019. Patterns of community assembly in the developing chicken microbiome reveal rapid primary succession. MicrobiologyOpen 8 (9), e00821.

Katsma, W.E.A., De Koeijer, A.A., Jacobs-Reitsma, W.F., Mangen, M.-J.J., Wagenaar, J. A., 2007. Assessing Interventions to Reduce the Risk of *Campylobacter* Prevalence in Broilers. Risk Anal. 27 (4), 863–876.

Kuhnke, D., Werner, N., Kreienbrock, L., 2020. Occurrence of ESBL-producing *Escherichia coli* in healthy, living food-producing animals in europe: a systematic review. CAB Rev.: Perspect. Agric. Vet. Sci. Nutr. Nat. Resour. https://doi.org/10.1079/pavsnnr202015010.

Laube, H., Friese, A., von Salviati, C., Guerra, B., Käsbohrer, A., Kreienbrock, L., Roesler, U., 2013. Longitudinal monitoring of extended-spectrum-beta-lactamase/AmpC-Producing *Escherichia coli* at German broiler chicken fattening farms. Appl. Environ. Microbiol. 79 https://doi.org/10.1128/AEM.00856-13.

Lu, J., Idris, U., Harmon, B., Hofacre, C., Maurer, J.J., Lee, M.D., 2003. Diversity and succession of the intestinal bacterial community of the maturing broiler chicken. Appl. Environ. Microbiol. 69 (11), 6816–6824.

Luyckx, K., Dewulf, J., Van Weyenberg, S., Herman, L., Zoons, J., Vervaet, E., Heyndrickx, M., De Reu, K., 2015a. Comparison of sampling procedures and microbiological and non-microbiological parameters to evaluate cleaning and disinfection in broiler houses. Poul. Sci. 94 (4), 740–749.

Luyckx, K.Y., Van Weyenberg, S., Dewulf, J., Herman, L., Zoons, J., Vervaet, E., Heyndrickx, M., De Reu, K., 2015b. On-farm comparisons of different cleaning protocols in broiler houses. Poul. Sci. 94 (8), 1986–1993.

Marshall, K., 2018. One Age, One Site. Poultry World. February 23, 2018. https://www.poultryworld.net/health-nutrition/one-age-one-site/ (accessed 16 October 2022).

Meijerink, N., Kers, J.G., Velkers, F.C., van Haarlem, D.A., Lamot, D.M., de Oliveira, J.E., Smidt, H., Stegeman, J.A., Rutten, V.P.M.G., Jansen, C.A., 2020. Early life inoculation with adult-derived microbiota accelerates maturation of intestinal microbiota and enhances NK cell activation in broiler chickens. Front. Vet. Sci. 7 (November), 584561.

Methner, U., Rösler, U., 2020. Efficacy of a competitive exclusion culture against extended-spectrum β-lactamase-producing *Escherichia coli* strains in broilers using a seeder bird model. BMC Vet. Res. 16 (1), 143.

Methner, U., Friese, A., Rösler, U., 2019. Competitive exclusion: a tool to combat extended-spectrum β-lactamase-producing *Escherichia coli* strains in chickens. Res. Vet. Sci. 123 (April), 124–128.

Mevius, D., Heederik, D., 2014. Reduction of antibiotic use in animals 'let's go Dutch. EFSA J. June, 9. https://doi.org/10.1007/s00003-014-0874-z.

Mevius, D., Heederik, D., Duijkeren, E., Veldman, K., van Essen, A., Kant, A., Liakopoulos, A., Geurts, Y., van Pelt, W., Mughini Gras, L., Schmitt, H., Dierikx, C., van Hoek, A., Evers, E., de Roda Husman, A., Blaak, H., van Dissel, J., Smid, J., Dohmen, W., … de Groot, J., 2018. Summary ESBL-Attribution-Analysis (ESBLAT). Https://Www.1health4food.Nl/En/Show/Chance-of-ESBL-Contamination-via-Livestock-Farming-Is-Small.Htm. February 2018. https://www.1health4food.nl/upload_mm/7/a/1/1155093f-8cd2–4dce-b19f-89e103990bcd_Summary%20ESBL_attribution%20_EN.pdf (accessed 28 February 2021).

Mezhoud, H., Chantziaras, I., Iguer-Ouada, M., Moula, N., Garmyn, A., Martel, A., Touati, A., Smet, A., Haesebrouck, F., Boyen, F., 2016. Presence of antimicrobial resistance in coliform bacteria from hatching broiler eggs with emphasis on ESBL/AmpC-producing bacteria. Avian Pathol. 45 (4), 493–500.

Mo, S.S., Kristoffersen, A.B., Sunde, M., Nødtvedt, A., Norström, M., 2016. Risk factors for occurrence of cephalosporin-resistant *Escherichia coli* in Norwegian broiler flocks. Prev. Vet. Med. 130 (August), 112–118.

Motola, G., Hafez, H.M., Brüggemann-Schwarze, S., 2020. Efficacy of six disinfection methods against extended-spectrum beta-lactamase (ESBL) Producing *E. coli* on eggshells in vitro. PloS One 15 (9), e0238860.

Mughini-Gras, L., Dorado-García, A., van Duijkeren, E., van den Bunt, G., Dierikx, C.M., Bonten, M.J.M., Bootsma, M.C.J., Schmitt, H., Hald, T., Evers, E.G., de Koeijer, A., van Pelt, W., Franz, E., Mevius, D.J., Heederik, D.J.J., ESBL Attribution Consortium, 2019. Attributable sources of community-acquired carriage of *Escherichia coli* containing β-Lactam antibiotic resistance genes: a population-based modelling study. Lancet Planet. Health 3 (8), e357–e369.

Nethmap-MARAN 2019. 2019. NethMap 2019: Consumption of Antimicrobial Agents and Antimicrobial Resistance among Medically Important Bacteria in the Netherlands / MARAN 2019: Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2018. June 27, 2019. https://www.wur.nl/en/show/MARAN-2019-rapport.htm (accessed 26 April 2021).

Nethmap-MARAN 2021. 2021. NethMap 2021: Consumption of Antimicrobial Agents and Antimicrobial Resistance among Medically Important Bacteria in the Netherlands / MARAN 2021: Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2020. June 2021. https://www.wur.nl/en/show/Nethmap-MARAN-2021.htm (accessed 4 April 2022).

Nuotio, L., Schneitz, C., Nilsson, O., 2013. Effect of competitive exclusion in reducing the occurrence of *Escherichia coli* producing extended-spectrum β-Lactamases in the ceca of broiler chicks. Poul. Sci. 92 (1), 250–254.

Oakley, B.B., Lillehoj, H.S., Kogut, M.H., Kim, W.K., Maurer, J.J., Pedroso, A., Lee, M.D., Collett, S.R., Johnson, T.J., Cox, N.A., 2014. The chicken gastrointestinal microbiome. FEMS Microbiol Lett. 360 (2), 100–112. https://doi.org/10.1111/1574-6968.12608. Epub 2014 Oct 13. PMID: 25263745.

Oikarainen, P.E., Pohjola, L.K., Pietola, E.S., Heikinheimo, A., 2019. Direct vertical transmission of ESBL/PAmpC-producing *Escherichia coli* limited in poultry production pyramid. Vet. Microbiol. 231 (April), 100–106.

Pacholewicz, E., Liakopoulos, A., Swart, A., Gortemaker, B., Dierikx, C., Havelaar, A., Schmitt, H., 2015. Reduction of extended-spectrum-β-Lactamase- and AmpC-β-lactamase-producing *Escherichia coli* through processing in two broiler chicken slaughterhouses. Int. J. Food Microbiol. 215 (December), 57–63.

Pitout, J.D., 2009. Recent changes in the epidemiology and management of extended-spectrum β-lactamase-producing enterobacteriaceae. F1000 Med. Rep. 1 (November) https://doi.org/10.3410/M1-84.

Plaza-Rodríguez, C., Correia Carreira, G., Käsbohrer, A., 2018. A probabilistic transmission model for the spread of extended-spectrum-β-lactamase and AmpC-

β-lactamase-producing *Escherichia coli* in the broiler production chain. Risk Anal. 38 (12), 2659–2682.

Projahn, M., Daehre, K., Roesler, U., Friese, A., 2017. Extended-spectrum-beta-lactamase- and plasmid-encoded cephamycinase- producing enterobacteria in the broiler hatchery as a potential mode of pseudo- vertical transmission. Appl. Environ. Microbiol. 83 (1) https://doi.org/10.1128/AEM.02364-16.

Reuland, E.A., Al Naiemi, N., Kaiser, A.M., Heck, M., Kluytmans, J.A., Savelkoul, P.H., Elders, P.J., Vandenbroucke-Grauls, C.M., 2016. Prevalence and risk factors for carriage of ESBL-producing enterobacteriaceae in Amsterdam. J. Antimicrob. Chemother. 71 (4), 1076–1082. https://doi.org/10.1093/jac/dkv441.

Sadeghi, M., Tavakkoli, H., Golchin, M., Ghanbarpour, R., Amanollahi, S., 2018. Efficacy and safety of poulvac *E. coli* vaccine in broiler chickens challenged with E. coli Serotype O78 and an acute field isolate. Comp. Clin. Pathol. 27 (6), 1629–1636.

C.B.S. Statline. 2022a. February 24, 2022. https://opendata.cbs.nl/statline/#/CBS/nl/dataset/80780ned/table?dl=4115 (accessed 29 June 2022).

C.B.S. Statline. 2022b. May 31, 2022. https://opendata.cbs.nl/statline/#/CBS/en/dataset/83474ENG/table?ts=1616407710127 (accessed 29 June 2022).

Swelum, A.A., Elbestawy, A.R., El-Saadony, M.T., Hussein, E.O.S., Alhotan, R., Suliman, G.M., Taha, A.E., Ba-Awadh, H., El-Tarabily, K.A., Abd El-Hack, M.E., 2021. Ways to minimize bacterial infections, with special reference to *Escherichia coli*, to cope with the first-week mortality in chicks: an updated overview. Poul. Sci. 100 (5), 101039.

Teunis, P.F.M., Evers, E.G., Hengeveld, P.D., Dierikx, C.M., Wielders, C.C.H., van Duijkeren, E., 2018. Time to acquire and lose Carriership of ESBL/PAmpC Producing *E. coli* in humans in the Netherlands. PloS One 13 (3), e0193834.

Toni, T., Welch, D., Strelkowa, N., Ipsen, A., Stumpf, M.P.H., 2009. Approximate bayesian computation scheme for parameter inference and model selection in dynamical systems. J. R. Soc. Interface 6 (31), 187–202.

Widgren, S., Bauer, P., Eriksson, R., Engblom, S., 2019. SimInf: An R package for data-driven stochastic disease spread simulations. J. Stat. Softw. 91 (12) https://doi.org/10.18637/jss.v091.i12.