HAL
open science

Impact of antimicrobial usage on the transmission dynamics of antimicrobial resistant bacteria among pigs

Emmanuel N. Abatih, Lis Alban, Annette K. Ersbøll, Danilo M. Lo Fo Wong

To cite this version:

Emmanuel N. Abatih, Lis Alban, Annette K. Ersbøll, Danilo M. Lo Fo Wong. Impact of antimicrobial usage on the transmission dynamics of antimicrobial resistant bacteria among pigs. Journal of Theoretical Biology, 2009, 256 (4), pp.561. 10.1016/j.jtbi.2008.10.017 . hal-00554520

HAL Id: hal-00554520

https://hal.science/hal-00554520v1

Submitted on 11 Jan 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Author's Accepted Manuscript

www.elsevier.com/locate/yjtbi

Impact of antimicrobial usage on the transmission dynamics of antimicrobial resistant bacteria among pigs

Emmanuel N. Abatih, Lis Alban, Annette K. Ersbøll, Danilo M. Lo Fo Wong

PII: S0022-5193(08)00544-4
DOI: doi:10.1016/j.jtbi.2008.10.017
Reference: YJTBI 5336

To appear in: *Journal of Theoretical Biology*

Received date: 29 May 2008
Revised date: 22 September 2008
Accepted date: 14 October 2008

Cite this article as: Emmanuel N. Abatih, Lis Alban, Annette K. Ersbøll and Danilo M. Lo Fo Wong, Impact of antimicrobial usage on the transmission dynamics of antimicrobial resistant bacteria among pigs, *Journal of Theoretical Biology* (2008), doi:10.1016/j.jtbi.2008.10.017

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting galley proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ACCEPTED MANUSCRIPT

Impact of antimicrobial usage on the transmission dynamics of antimicrobial resistant bacteria among pigs

Emmanuel N. Abatih [a,*], Lis Alban [b], Annette K. Ersbøll [a], Danilo M. Lo Fo Wong [c]

[a] *University of Copenhagen, Faculty of Life Sciences, Department of Large Animal Sciences, Unit of Epidemiology, Grønnegaardsvej 8, 1870 Frederiksberg C, Denmark*

[b] *Danish Meat Association, Vinkelvej 11, 8620 Kjellerup, Denmark*

[c] *World Health Organization, Department of Food Safety,Zoonoses and Food-borne Diseases, Avenue Appia 20, CH - 1211,Geneva 27, Switzerland*

Accepted manuscript

Abstract

There is increasing evidence showing that antimicrobial consumption provides a powerful selective force that promotes the emergence of resistance in pathogenic, commensal as well as zoonotic bacteria in animals. The main aim of this study was to develop a modeling framework that can be used to assess the impact of antimicrobial usage in pigs on the emergence and transmission of resistant bacteria within a finisher pig farm. The transmission dynamics of drug-sensitive and drug-resistant bacteria among pigs in the herd were characterized by studying the local and global stability properties of steady state solutions of the system. Numerical simulations demonstrating the influence of factors such as initial prevalence of infection, presence of pre-existing antimicrobial resistant mutants, and frequency of treatment on

ACCEPTED MANUSCRIPT

predicted prevalence were performed. Sensitivity analysis revealed that two parameters had a huge influence on the predicted proportion of pigs carrying resistant bacteria: a) the transmission coefficient between uninfected pigs and those infected with drug-resistant bacteria during treatment (β_2) and after after treatment stops (β_3), and b) the spontaneous clear-out rate of drug-resistant bacteria during treatment (γ_2) and immediately after treatment stops (γ_3). Control measures should therefore be geared towards reducing the magnitudes of β_2 and β_3 or increasing those of γ_2 and γ_3.

Key words: antimicrobial resistance; epidemic model; stability analysis; pigs; time varying treatment effect.

Accepted manuscript

1 Introduction

There is increasing evidence showing that antimicrobial usage provides a powerful selective force that promotes the emergence of resistance in both pathogenic, commensal and zoonotic bacteria in animals (Barbosa and Levy, 2000; Angulo et al., 2004; Asai et al., 2000; Delsol et al., 2005; Jensen et al., 2006). The emergence, persistence and spread of resistant bacteria is of growing concern since it might compromise the control of infections by reducing treatment options for infected animals. This may lead to an overall increase in disease transmission, morbidity, mortality and sometimes to economic losses to the animal production industry where tonnes of antimicrobial agents are consumed yearly (McGowan, 2001).

Most foodborne zoonotic infections are caused by *Salmonella*, *Campylobacter*

* Corresponding author. Tel: 004535333093; fax: 004535333022
Email address: `ena@life.ku.dk` (Emmanuel N. Abatih).

ACCEPTED MANUSCRIPT

and pathogenic *E. coli* residing in the gut micro-flora of animals (Altekruse et al., 1999; Engberg et al., 2006; McDermott, 2006). *Salmonella* has main reservoirs in cattle, pig, chicken and turkey, *Campylobacter spp.*(especially jejuni and coli) has reservoirs in chicken and turkey while pathogenic *E. coli* has main reservoir in cattle (Angulo et al., 2004). Antimicrobial usage in animals increases the frequency of resistant genes in target bacteria as well as in commensal and zoonotic bacteria (via horizontal gene transfer). Bacteria carrying these resistant genes can be transferred to humans via the food chain (White and McDermott, 2006; Teale, 2004). Since the farm is the only point along the food chain where antimicrobial agents are prescribed and used, an assessment of the impact of antimicrobial usage in animals on the within–herd transmission dynamics of resistant bacteria (or their determinants) is key to limiting the emergence and spread of resistant bacteria within the herd and consequently the amount of resistant bacteria input to the food chain (Delsol et al., 2005).

Very few attempts have been made to assess the impact of antimicrobial consumption on the transmission dynamics of resistant bacteria among animals (Kavanagh et al., 2005). The few studies that have characterized the dynamics of resistant genes and bacteria and the effect of antimicrobial usage have been in human populations in Intensive Care Units or closed communities. For example, Austin et al. (1997) proposed a mathematical modeling framework for studying the relationship between resistance in commensal organisms and antibiotic consumption. Their model characterized the colonization of a human host population (in an intensive care unit) by commensal bacteria in the presence of antimicrobial agents. Similar models have also been defined and used to study the transmission dynamics of resistance in commensal bacteria in humans (Levin and Andreason, 1999; Lipsitch and Samore, 2002; Smith

Accepted manuscript

ACCEPTED MANUSCRIPT

et al., 2002). These models assumed that the treatment effect was constant over time. However, due to the fluctuations in drug concentration over time resulting from absorption, distribution and elimination in the animal, it appears more appropriate to assume that the treatment effect is time-varying (Huang et al., 2003; Rong et al., 2007). Lower resistance selection intensities are known to be associated with intramuscular injections as compared to oral drug administration in animals (Wiuff et al., 2003; Bibbal et al., 2007). However, when large groups of animals are treated, the most common mode of administration is oral, via medicated feed or water. This study focuses on pigs which account for the majority of antibiotic consumption in Denmark as a result of pigs being the largest part of the livestock production industry (Anonymous, 2007).

There is a need for a modeling framework based on current knowledge of the epidemiology of antimicrobial resistance in animals and on important factors which can be used to characterize the dynamics of resistant bacteria in animals in the herd under the influence of antimicrobial pressure. Knowledge of the impact of antimicrobial usage in animals on the transmission dynamics of resistant bacteria will improve the understanding of the influence of on-site farming practices on the dynamics of resistance in bacteria in animals and will also form the basis for a release assessment (component of a risk assessment).

The main aim of this study was to develop a modeling framework that can be used to assess the impact of antimicrobial usage amongst pigs on the emergence and transmission of resistant bacteria within a finisher pig herd. Some simplifying assumptions were used in the model building process as described in section 2. This section also discusses threshold parameters and the two treatment strategies investigated; first-line (*recommended drug*) and episodic

Accepted manuscript

ACCEPTED MANUSCRIPT

treatments (first-line followed by second-line). Transmission dynamics of drug-sensitive and drug-resistant bacteria among pigs in the herd were characterized by studying the local and global stability properties of steady state solutions of the system under first-line and episodic treatments as discussed in section 3. Results of numerical simulations demonstrating the influence of factors such as initial prevalence of infection, presence of pre-existing antimicrobial resistant mutants, and frequency of treatment on predicted prevalence are also presented. In addition, the estimation of the fraction of pigs with drug-resistant bacteria in the herd prior to transport to slaughter for various levels of antimicrobial consumption is discussed. This section ends with a discussion of the results of the uncertainty and sensitivity analyzes. A discussion of the main findings and some future perspectives are presented in section 4.

2 Material and Methods

A deterministic model is presented to give an average of the processes governing the dynamics of the spread of resistant bacteria amongst pigs in a finisher herd. A herd was assumed to consist of pigs assigned to different states based on their infection status. They can be either uninfected (susceptible) (S), predominantly infected with drug-sensitive bacteria (I_I) or predominantly infected with drug-resistant bacteria (I_R). Some simplifying assumptions of the model are described followed by the model building process in which the various transitions to and from each state are discussed. A discussion of a function which describes the time-varying treatment effect is presented and threshold parameters governing the dynamics of resistant bacteria within the pig population are derived.

Accepted manuscript

ACCEPTED MANUSCRIPT

2.1 Assumptions:

The model is based on the following assumptions which were made in order to improve understanding of the dynamics of resistant bacteria among pigs in the herd (Ivanek et al., 2004).

- During treatment, all animals are treated and there are no deaths, be it infection-related or natural.
- There is random contact between pigs in the herd. No predefined pattern and contact may or may not lead to infection.
- Once an animal becomes infected, it immediately becomes infectious to other animals. We thus ignore the time delay between acquisition of infection and becoming infectious.
- Treatment does not offer immunity against infection, thus after successful treatment the animal recovers from the infection and immediately becomes susceptible again.
- It was assumed that, after treatment stops, pigs with drug-resistant bacteria spontaneously clear-off bacteria faster than those with drug-sensitive bacteria do but rather have a slower transmission rate due to fitness cost incurred by antimicrobial resistance(Banhoeffer et al. 1997; Lipsitch and Samore, 2002).
- Drug is assumed to have no effect on pigs with resistant bacteria and offers a metaphylactic advantage to uninfected pigs.
- Pharmacokinetic properties such as volume of distribution and elimination rate of the drug are ignored. Only pharmacodynamic properties are considered. The daily recovery rate is assumed to be proportional to the quantity of antimicrobial agent (dose) used.

Accepted manuscript

ACCEPTED MANUSCRIPT

The implications of relaxing one or more of these assumptions will be discussed.

2.2 Model formulation

Uninfected pigs enter the infected class at a per capita rate of $\beta_1 I_I$, with β_1 denoting the transmission rate parameter and given by the contact rate multiplied by the probability of infection per contact and I_I, the number of infected pigs. In a similar manner, uninfected pigs acquire resistance and move into the drug-resistant class at a per capita rate of $\beta_2 I_R$ with β_2 given by the contact rate between pigs infected with susceptible bacteria and those infected with drug–resistant bacteria multiplied by the probability of infection per contact. Bacteria in infected pigs acquire drug resistance through plasmid transfer at rate αI_I with α denoting the product of the contact rate between pigs infected with drug–sensitive bacteria and those with drug–resistant bacteria and the probability of infection per contact. The above explanations are all based on the mass action principle (Hethcote, 2000). Following antimicrobial treatment, infected pigs recover from the infection at a time-varying recovery rate of $\eta(t)$ or due to spontaneous kick-out of pathogenic bacteria at rate γ_1. Pigs infected with resistant bacteria may spontaneously clear off bacteria at rate γ_2 and move into the uninfected class. Pigs with drug–sensitive bacteria also develop resistance via amassed spontaneous mutations at the rate of ϕ per day.

The flux of pigs from one state to the other is as shown on Fig. 1. The total population at any time t is assumed to be constant i.e. no introduction of new finishers or removal until transport to slaughter. The herd is said to be at an adiabatic state and thus:

Accepted manuscript

ACCEPTED MANUSCRIPT

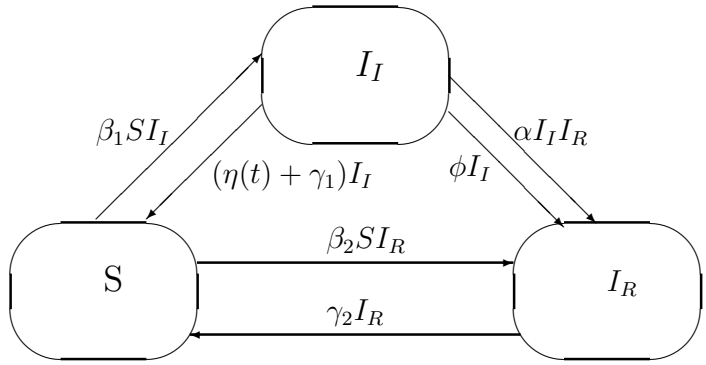


Fig. 1. Flow of pigs from one state to another. S stands for uninfected pigs, I_I for infected and drug-sensitive pigs and I_R for infected and drug-resistant pigs.

$$S(t) + I_I(t) + I_R(t) = N, \quad \forall\, t \geq 0 \tag{1}$$

The dynamics of the spread of resistance within the herd can be described by the following set of coupled differential equations:

$$\begin{aligned} S' &= -\beta_1 S(t)\frac{I_I(t)}{N} - \beta_2 S(t)\frac{I_R(t)}{N} + (\eta(t)+\gamma_1)I_I(t) + \gamma_2 I_R(t) \\ I_I' &= \beta_1 S(t)\frac{I_I(t)}{N} - \alpha I_I(t)\frac{I_R(t)}{N} - (\eta(t)+\gamma_1)I_I(t) - \phi I_I(t) \\ I_R' &= \beta_2 S(t)\frac{I_R(t)}{N} + \alpha I_I(t)\frac{I_R(t)}{N} + \phi I_I(t) - \gamma_2 I_R(t) \\ S(0) &= S_0 > 0,\ I_I(0) = I_{I_0} > 0,\ \textit{and}\ I_R(0) = I_{R_0} \geq 0. \end{aligned} \tag{2}$$

The parameters ϕ, γ_1, γ_2, which are positive real constants, characterize the flow of pigs from one state to another. These parameters can be described as representing exponentially distributed waiting times in the states involved. For example, γ_1 can be interpreted as the probability of residing in the infected state t days after movement into this state due to spontaneous clear-off by pigs of drug-sensitive bacteria and is given by $P(t) = e^{\gamma_1 t}$ with $\frac{1}{\gamma_1}$ as the mean waiting time in this state (Hethcote, 2000).

Accepted manuscript

ACCEPTED MANUSCRIPT

Rather than working with the population as a whole, fractions were used so that the force of infection, which is the per capita rate at which susceptible pigs contract the infection (e.g. $\beta_1 \frac{I_I(t)}{N}$) becomes independent of the population size (Hethcote, 2000). Setting $x = \frac{S(t)}{N(t)}$, $y = \frac{I_I(t)}{N(t)}$ and $z = \frac{I_R(t)}{N(t)}$ we have the following system of equations:

$$\begin{aligned} \frac{dx}{dt} &= -\beta_1 xy - \beta_2 xz + (\eta(t) + \gamma_1)y + \gamma_2 z \\ \frac{dy}{dt} &= \beta_1 xy - \alpha yz - (\eta(t) + \gamma_1 + \phi)y \\ \frac{dz}{dt} &= \beta_2 xz + \alpha yz + \phi y - \gamma_2 z \\ &\textit{where} \;\; x + y + z = 1, \;\; \forall\, t \geq 0 \\ x(0) = x_0 > 0,\; y(0) = y_0 > 0,\; &\textit{and}\; z(0) = z_0 \geq 0. \end{aligned} \tag{3}$$

Given the constraint $1 = x + y + z$, system (3) can be reduced to the following system of two equations :

$$\begin{aligned} \frac{dy}{dt} &= \beta_1(1 - y - z)y - \alpha yz - (\eta(t) + \gamma_1 + \phi)y \\ \frac{dz}{dt} &= \beta_2(1 - y - z)z + \alpha yz + \phi y - \gamma_2 z \\ &\textit{with} \;\; x = 1 - y - z, \;\; \forall\, t \geq 0 \\ x(0) = x_0 > 0,\; y(0) = y_0 > 0,\; &\textit{and}\; z(0) = z_0 \geq 0. \end{aligned} \tag{4}$$

2.3 Treatment of infected pigs

Since our analysis is focused on finisher pigs, it was assumed that the age and weight are approximately the same for each of the pigs. It was also assumed that the daily recovery rate is directly proportional to the drug concentration

Accepted manuscript

ACCEPTED MANUSCRIPT

at the infection site. This implies that only concentration dependent drugs such as fluoroquinolones and amino-glycosides such as gentamicin and streptomycin could be considered. During therapy, the change in average treatment effect against time was modeled based on the bi-exponential model (Wong et al., 1979; Greenblatt et al., 2002; Davies et al., 2006):

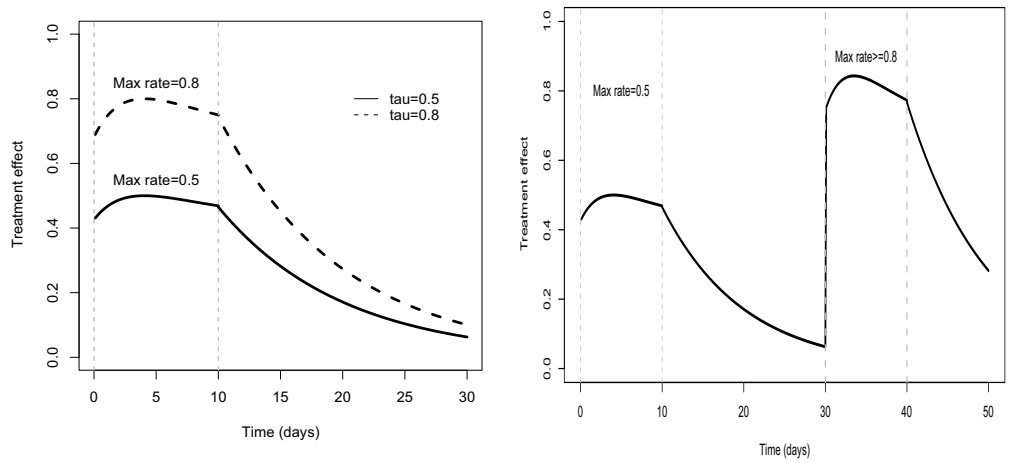
$$\eta(t^*) = \tau * exp(-A * t^*) - \tau * exp(-B * t^*) \tag{5}$$

A and B are parameters usually estimated from concentration-time data points and t^* is the time (in days) during treatment. Arbitrary values were assumed for A and B to depict behavior typical of concentration-dependent drugs. Fig. 2(a) depict the change in treatment effect with time for a concentration-dependent drug with recovery rates of 0.5 and 0.8 respectively. The infected pigs were expected to recover after approximately 3 days when the recovery rate was 0.33, 2 days with recovery rate of 0.5 and 1.25 days with recovery rate of 0.8. It was assumed that immediately after treatment stopped, the treatment effect decreased exponentially until it hit zero. Equation 5 was modified for each recovery rate such that the maximum attainable effects were obtained.

In the event of treatment failure following the first-line treatment regime for a given number of days, an alternative therapy could be applied. Fig. 2(b) shows the situation where first-line therapy was followed by second-line therapy (increase in dose for current treatment or use of an alternative, more effective drug). By considering the recovery rates only, any drug can easily be modeled ignoring its pharmacodynamic and pharmacokinetic properties.

Accepted manuscript

ACCEPTED MANUSCRIPT



(a) First-line treatment

(b) Episodic treatment

Fig. 2. a): Single treatment effect (η) as a function of the recovery rate (τ) and time. After treatment stops, the effect decreases exponentially with time. b): Episodic treatment effect as a function of the recovery rate and time. The first-line treatment with recovery rate $\tau = 0.5$ was followed by a second-line treatment with recovery rate $\tau = 0.8$ after 20 days

2.4 Threshold parameters

The transmission dynamics of any infection in the herd is governed by the basic reproduction number (R_0) which is defined as the expected number of secondary infected pigs resulting from the introduction of one infected pig in an entirely susceptible pig population in the herd (Hethcote, 2000; Edelstein-Keshet, 2005). It is given by the product of the rate at which newly infected pigs arise and the average infectious period. For pigs with drug-sensitive bacteria $R_i = \beta_1/(\eta(t) + \phi + \gamma_1)$ and for those with drug–resistant bacteria $R_r = (\beta_2 + \alpha)/\gamma_2$. It can be immediately seen that R_i is dependent on the treatment effect η whereas R_r is not.

In order for an infection to be established in the pig population in the herd, $\frac{dy}{dt} > 0$ which leads to the following inequality:

$$x > \frac{\eta(t) + \gamma_1 + \phi + \alpha * z}{\beta_1} > \frac{\eta(t) + \gamma_1 + \phi}{\beta_1} = \frac{1}{R_i}$$

Accepted manuscript

ACCEPTED MANUSCRIPT

For an infection free herd, x=1, y=0 and z=0 from which it follows that for an infection to be established, R_i must exceed 1. Otherwise, the infection will vanish from the finisher pig farm with time.

For antimicrobial resistance to persist, $\frac{dz}{dt} > 0$ i.e.

$$\beta_2 xz + \alpha yz + \phi y - \gamma_2 z > 0$$

which is true only if $\beta_2 x - \gamma_2 > 0$ or $\alpha y - \gamma_2 > 0$. Basic algebraic manipulations (and using the implication : $\beta_2 x > \gamma_2 \Rightarrow \beta_2 x + \alpha x > \gamma_2$ since $\alpha > 0$ and $x > 0$ or $\alpha y > \gamma_2 \Rightarrow \alpha y + \beta_2 y > \gamma_2$ since $\beta_2 > 0$ and $y > 0$) yield that the inequality holds true if $x > \frac{1}{R_r}$ or $y > \frac{1}{R_r}$. Combining these two inequalities (using the property of additivity of inequalities), and using the equation $x + y = 1$ (for resistant-infection free herd), it follows that $R_r > 1$. Thus for pigs carrying antimicrobial resistant bacteria to persist in the herd, $R_r > 1$.

3 Analysis of system (3)

3.1 Boundedness of solutions

The boundedness of the solutions of system (3) guarantees its mathematical and epidemiological validity over the entire duration of stay of the pigs in the herd prior to transport to slaughter (Esteva and Vargas 1998). To show that system (3) is bounded, it suffices to show that the region, $\Omega = \{(x, y, z : \ x > 0, \ \ y > 0, \ \ z \geq 0, \ \ R_i \geq 0, \ \ R_r \geq 0, \ \ and \ \ x + y + z = 1\}$ is positive invariant. This is trivial since for all $t > 0$ the solution paths of system (3) all fall in Ω i.e. the vector field of the boundary does not point to the exterior. Therefore

Accepted manuscript

ACCEPTED MANUSCRIPT

system (3) is mathematically and epidemiologically well posed.

3.2 Existence and stability of equilibria

Equilibria (steady states) represent behavior that persists in time for the system and were obtained by setting the right hand side (r.h.s) of all the equations in system (3) to zero. The following steady state solutions were obtained : infection free steady state given by $E_0 = (1, 0, 0)$ which exists for all parameter values, resistant free steady state $E_1 = (x^+, y^+, 0)$ which makes sense biologically for $R_i > 0$, the resistant steady state $E_2 = (x^-, 0, z^-)$ which exists for $R_r > 1$ and the non-trivial co-existence steady state $E_3 = (x^*, y^*, z^*)$ which exists for $R_i > 1$, $\beta_2 < \alpha$ and $R_r < 1$.

By local stability is meant the convergence of any solution of the system in the neighborhood of a steady state to the steady state whereas global stability implies convergence of solutions anywhere in the region Ω to the steady state. Local stability is studied by linearizing the system around the steady state and global stability by defining a Lyapunov function (Esteva and Vargas, 1998; Hethcote, 2000; Korobeinikov, 2006). Because we assume a bilinear incidence rate (mass action), the Lyapunov functions defined are based on those given by Korobeinikov (2006).

Accepted manuscript

3.2.1 Stability of infection-free equilibria

The trivial infection free equilibrium is often of interest since it is the desired equilibrium state. It reflects successful treatment where neither pigs with sensitive nor resistant bacteria persist in time.

ACCEPTED MANUSCRIPT

The local asymptotic stability of $E_0 = (1, 0, 0)$ can be studied via the reduced steady state $E_{00} = (0, 0)$ and is determined by the signs of the eigen values of the Jacobian matrix obtained by linearizing system (4) around E_{00}. The Jacobian represents the best linear approximation of system (4) at $E_{00} = (0, 0)$. The following Jacobian matrix was obtained:

$$\mathbf{J}(E_{00}) = \begin{pmatrix} m_{11} & 0 \\ \phi & m_{22} \end{pmatrix}$$

where $m_{11} = \beta_1(1 - \frac{1}{R_i})$ and $m_{22} = \beta_2 - \gamma_2$

The eigen values are given by the roots of the characteristic equation:

$$det(\mathbf{J}(E_{00}) - \lambda\mathbf{I}) = 0$$

where $\mathbf{I}$ is the 2×2 identity matrix. This gives:

$$(m_{11} - \lambda)(m_{22} - \lambda) = 0$$

from which the eigen values were: $\lambda_1 = m_{11} = \beta_1(1 - \frac{1}{R_i})$ and $\lambda_2 = m_{22} = \beta_2 - \gamma_2$.

This steady state will be locally asymptotically stable if $\lambda_1 < 0$ and $\lambda_2 < 0$. $\lambda_1 < 0$ if $R_i < 1$ and $\lambda_2 < 0$ if $\frac{\beta_2}{\gamma_2} < 1$. But then, $\frac{\beta_2}{\gamma_2} = R_r - \frac{\alpha}{\gamma_2} \Rightarrow R_r < 1 + \frac{\alpha}{\gamma_2}$ from which it follows $R_r < 1$.

Thus, if pigs infected with drug-sensitive and drug-resistant bacteria arise at a rate lower than the rate at which they loss their infection status, then the infection will not invade the pig population. This means therefore that any solution of system (3) in the neighborhood of (1,0,0) will approach the

Accepted manuscript

ACCEPTED MANUSCRIPT

infection-free equilibrium.

The global stability of the infection-free steady state is established in the following proposition:

Proposition 1.1: All solutions in the region Ω approach the infection-free equilibrium $E_0 = (1, 0, 0)$ for $R_i \leq 1$ and $R_r \leq 1$.

Proof:

Consider the Lyapunov function: $V : \Omega \to R$, $V = y + z$

which is well defined and continuous in Ω. V=0 at $(x, y, z) = (1, 0, 0)$ and positive if $(x, y, z) \neq (1, 0, 0)$. Also for any solution $\phi(t)$, $V(\phi(t_0)) \geq V(\phi(t_1))$ for $t_1 > t_0$, where $\phi(t_j) \in V((x_0, y_0, z_0)) \setminus (x_0, y_0, z_0)$. The Lyapunov derivative of V is given by:

$$\dot{V} = \dot{y} + \dot{z}$$

$$= [\beta_1 x - (\eta(t) + \gamma_1 + \phi)]y + [\beta_2 x - \gamma_2]z + \phi y$$

On Ω, we have that $\beta_2 x - \gamma_2 \leq (\alpha + \beta_2)x - \gamma_2$, so

$$\dot{V} \leq (\eta(t) + \gamma_1 + \phi)[R_i x - 1]y + [(\beta_2 + \alpha)x - \gamma_2]z + \phi y$$

$$= (\eta(t) + \gamma_1 + \phi)[R_i x - 1]y + \gamma_2[(R_r x - 1]z + \phi y \leq 0$$

for $R_i \leq 1$ and $R_r \leq 1$

If $R_i < 1$ and $R_r < 1 : \dot{V} = 0$ iff $y = 0$ and $z = 0$

If $R_i = 1$ and $R_r = 1 : \dot{V} = 0$ iff $x = 1$ and $y = 0$

It thus follows from the Lyapunov-Lasalle (LaSalle, 1976) theorem that E_0

Accepted manuscript

ACCEPTED MANUSCRIPT

is globally asymptotically stable in Ω and so every trajectory of system (3) approaches the infection-free equilibrium for $R_i \leq 1$ and $R_r \leq 1$. E_0 is the unique steady state for system (3) when $R_i \leq 1$ and $R_r \leq 1$.

3.2.2 Co-existence (endemic-resistant) steady state

The previous steady state solution represented a scenario where neither pigs with drug-sensitive nor drug-resistant bacteria persisted in time. The non-trivial steady state, where both uninfected pigs, those with drug-sensitive and drug-resistant bacteria co-exist is denoted by $E_3 = (x^*, y^*, z^*)$ where for system (3)

$$x^* = 1 - y^* - z^*, \qquad y^* = \frac{(R_i - 1)}{R_i} - (1 + \frac{\alpha}{\beta_1})z^* \quad \text{and}$$

$$z^*_{1,2} = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

with $\quad a = (\beta_2 - \alpha)\frac{\alpha}{\beta_1} - \alpha, \;\; b = \frac{(\beta_2 - \alpha)}{R_i} - \phi(\frac{\alpha}{\beta_1} + 1) + (\alpha - \gamma_2) \;\; \text{and}$

$$c = \phi(\frac{(R_i - 1)}{R_i})$$

z^* is real and positive only if $a < 0$, $b < 0$, $b < \sqrt{b^2 - 4ac}$, and $c > 0$. $a < 0$ if $\beta_2 < \alpha$; $b < 0$ if $\beta_2 < \alpha$ and $R_r < 1$ and $c > 0$ if $R_i > 1$. E_3 is thus defined on the region Ω.

The variational (stability) matrix of system (4) at the reduced steady state, $E_{33} = (y^*, z^*)$ is given by:

$$\mathbf{J}(E_{33}) = \begin{pmatrix} m_{11} & m_{12} \\ m_{21} & m_{22} \end{pmatrix}$$

Accepted manuscript

ACCEPTED MANUSCRIPT

where $m_{11} = \beta_1[(\frac{\alpha}{\beta_1}+1)z^* - (\frac{(R_i-1)}{R_i})]$, $m_{12} = (\alpha+\beta_1)[(\frac{\alpha}{\beta_1}+1)z^* - (\frac{(R_i-1)}{R_i})]$, $m_{21} = (\alpha-\beta_2)z^* + \phi$ and $m_{22} = (\beta_2-\gamma_2)+(\beta_2-\alpha)[(\frac{\alpha}{\beta_1}+1)z^* - (\frac{(R_i-1)}{R_i})] - 2\beta_2 z^*$

The eigen values of $\mathbf{J}(E_{33})$ satisfy: $det(\mathbf{J}(E_{33}) - \lambda\mathbf{I}) = 0$, from which the following characteristic equation is obtained:

$$\lambda^2 + A_1\lambda + A_2 = 0$$

where $A_1 = -m_{11} - m_{22}$, and $A_2 = m_{11}m_{22} - m_{12}m_{21}$

It follows that $\lambda_1 = \frac{-A_1+\sqrt{A_1^2-4A_2}}{2}$ and $\lambda_2 = \frac{-A_1-\sqrt{A_1^2-4A_2}}{2}$

λ_1 and λ_2 are real if and only if $A_1^2 - 4A_2 > 0$. This holds true if $A_2 < 0$. $A_2 < 0$ if and only if $R_i > 1$, $R_r < 1$, and $\beta_2 < \alpha$. It immediately follows that $A_1 < \sqrt{A_1^2 - 4A_2}$. Also, $A_1 > 0$ for $\alpha < \beta_1$, $R_i > 1$, and $R_r < 1$. Therefore, the eigen values are all real but have opposite signs. This leads to an unstable steady state called saddle point. This steady state has two manifolds, one stable (corresponding to the negative eigen value) and the other unstable (corresponding to the positive eigen value). For the saddle point, the global stability was not investigated further.

Stability analysis of the resistant-free steady state ($E_1 = (x^+, y^+, 0)$) revealed that local asymptotic stability holds only if $R_i > 1$, $R_r < 1$, and $\alpha < \beta_2$ and global asymptotic stability holds provided that $R_i \geq 1$, $R_r \leq 1$ and $\alpha \leq \beta_2$. Also, for the resistant steady state ($E_2 = (x^-, 0, z^-)$) to be locally asymptotically stable, $R_i < 1$ and $R_r > 1$ and for global asymptotic stability, $R_i \leq 1$ and $R_r \geq 1$ (see Appendix). Table (1) summaries the results of the stability analysis.

Accepted manuscript

ACCEPTED MANUSCRIPT

Table 1
Existence of steady states for system (3) and conditions for their local and global asymptotical stability

Steady state	**Existence**	**LAS**	**GAS**
$E_0 = (1, 0, 0)$	Always	$R_i < 1$ & $R_r < 1$	$R_i \leq 1$ & $R_r \leq 1$
$E_1 = (x^+, y^+, 0)$	$R_i > 1$	$R_i > 1$, $R_r < 1$ & $\alpha < \beta$	$R_i \geq 1$, $R_r \leq 1$ & $\alpha \leq \beta_2$
$E_2 = (x^-, 0, z^-)$	$R_r > 1$	$R_i < 1$ & $R_r > 1$	$R_i \leq 1$ & $R_r \geq 1$
$E_3 = (x^*, y^*, z^*)$	$R_i > 1$,$R_r < 1$, & $\beta_2 < \alpha$	Unstable	NA

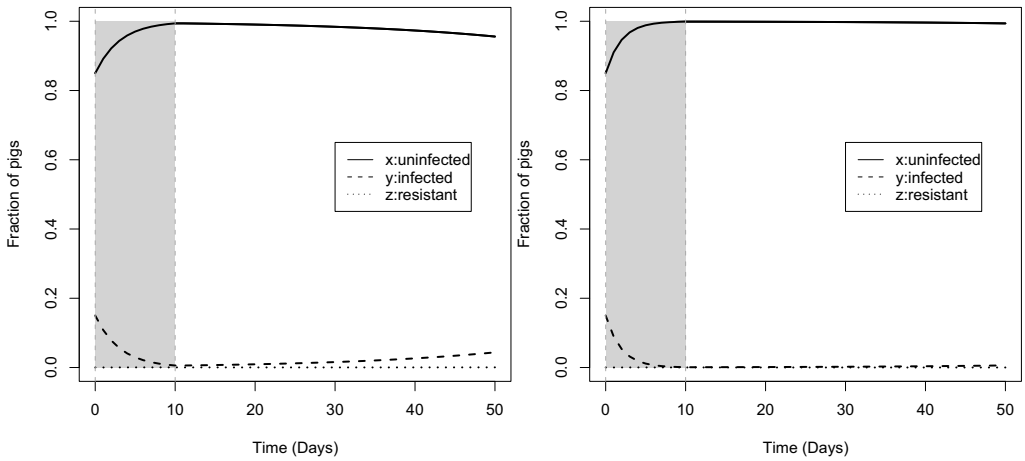
LAS=Local Asymptotic Stability; GAS=Global asymptotic stability; NA: Not Assessed

3.3 Numerical simulations

Numerical simulations were performed to illustrate the analytical findings and also to assess the effects of first-line and episodic treatments on the transmission dynamics of drug-sensitive and drug- resistant bacteria among pigs within a finisher herd. Infections in pigs may arise at any time point during their stay in the herd but in this study a time frame of 50 days prior to transport to slaughter was considered. Transmission parameter values were assumed in order to generate varying threshold parameters used to investigate the analytical results of system (3). In all the simulations, for a treatment with recovery rate 0.3, the effect varied according to: $\eta(t^*) = c_1 + 0.3 * exp(-0.2 * t^*) - 0.3 * exp(-0.3 * t^*)$ and with recovery rate 0.5: $\eta(t^*) = c_2 + 0.5 * exp(-0.2 * t^*) - 0.5 * exp(-0.3 * t^*)$. The parameters $c_1 = 0.26$ and $c_2 = 0.43$ were chosen such that the maximum attainable treatment effects were approximately 0.3 and 0.5 respectively. The fitness cost incurred by antimicrobial resistance in pigs was reflected in the simulations by a reduced transmission coefficient between pigs infected with drug-resistant bacteria and

Accepted manuscript

ACCEPTED MANUSCRIPT



(a) $\tau = 0.3$ with treatment effect $\eta \leq 0.3$ (b) $\tau = 0.5$ with treatment effect $\eta \leq 0.5$

Fig. 3. Solution trajectories for system (3) with initial proportions : $x_0 = 0.85$, $y_0 = 0.15$, $z_0 = 0$ and selected parameter values: $\beta_1 = 0.1$, $\beta_2 = 0.01$, $\alpha = 0.001$, $\gamma_1 = 0.04$, $\gamma_2 = 0.04$, *and* $\phi = 0.001$. The shaded region represents the treatment window. The infection was found to persist following treatment with recovery rate $\tau = 0.3$ (a) with complete eradication with recovery rate $\tau = 0.5$ (b). During treatment, in (a) $R_i = 0$ and $R_r = 0.28$ and after treatment $0.34 \leq R_i \leq 2.17$ and $R_r = 0.10$. In (b), during treatment $R_i = 0$ and $R_r = 0.28$ whereas after treatment $0.22 \leq R_i \leq 2.02$ and $R_r = 0.10$

those infected with drug-sensitive bacteria (α_2). A faster recovery rate was assumed for pigs with drug-resistant bacteria immediately after treatment stopped (γ_3). Lastly, since it was assumed that during treatment, the susceptibles were resistant to colonization by drug-sensitive bacteria but not by drug-resistant bacteria, the transmission coefficient between susceptible pigs and those carrying drug-sensitive bacteria ,β_1, was assumed to be zero during treatment.

Accepted manuscript

3.3.1 No pre-existing mutants: First-line treatment

Dynamics of pigs in the herd were studied for first-line treatments with varying recovery rates; 0.3, 0.5 and 0.8 respectively and for different initial proportion of infected pigs. It was assumed that prior to treatment, the proportion of pigs infected with resistant bacteria was zero i.e. $z_0 = 0$. It can be seen from Fig. 3a that following treatment with a daily recovery rate of 0.3, the proportion

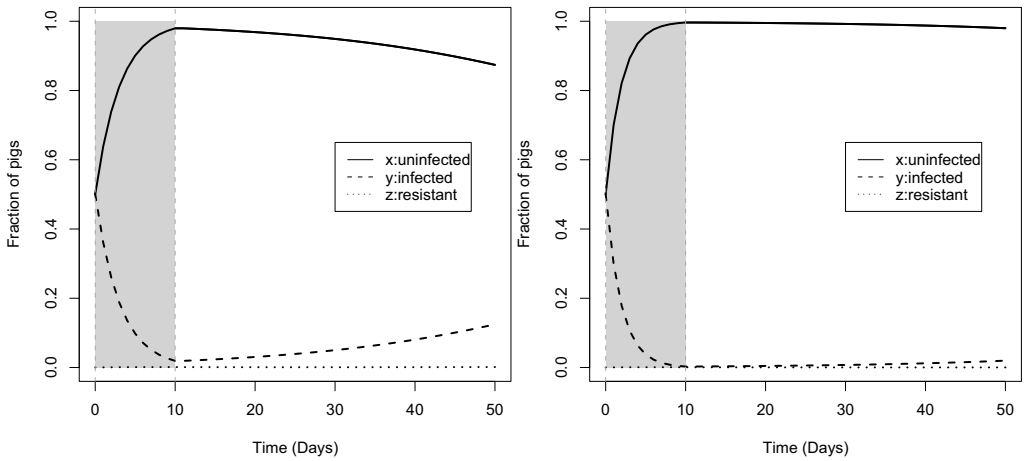
ACCEPTED MANUSCRIPT

of infected pigs decreased to about 1%. However, the infection started re-emerging 10 days post treatment. At the end of the study period, there were no pigs infected with resistant bacteria but about 4% of pigs infected with drug-sensitive bacteria persisted in the herd. This represented a significant reduction from the initial 15% infected prior to treatment. On the other hand, under the same conditions, for a drug with an average daily recovery rate of 0.5, no pigs infected with drug-sensitive bacteria re-emerged post treatment. The proportion of pigs infected with drug-sensitive bacteria was zero at the end of the study period compared to the initial prevalence of 15% (Fig. 3b). If prior to treatment, 50% of the pigs were infected with drug-sensitive bacteria, a treatment with a recovery at rate of 0.3 led to a higher proportion of infected pigs (about 12%) compared with that of a daily recovery rate of 0.5 (about 2%) prior to transport to slaughter (Figs. 4a and 4b). There were no pigs infected with drug-sensitive or drug-resistant bacteria following treatment with recovery rate=0.8 prior to transport to slaughter (not shown). Overall, It was observed that a higher initial proportion of pigs infected with drug-sensitive bacteria led to a higher proportion of pigs still carrying drug-sensitive bacteria prior to transport to slaughter.

Accepted manuscript

In all the previous simulations, it was assumed that $R_r < 1$ for which pigs with drug-resistant bacteria did not surface in the herd. The case where pigs with drug-resistant bacteria persisted prior to transport to slaughter ($R_r > 1$) was also considered. Almost 2% of the total pig population possessed drug-resistant bacteria prior to transport to slaughter with a daily recovery rate of 0.3 compared to about 1% for a treatment with daily recovery rate of 0.5 (Figs. 5 a and b).

ACCEPTED MANUSCRIPT



(a) $\tau = 0.3$ with treatment effect $\eta \leq 0.3$ (b) $\tau = 0.5$ with treatment effect $\eta \leq 0.5$

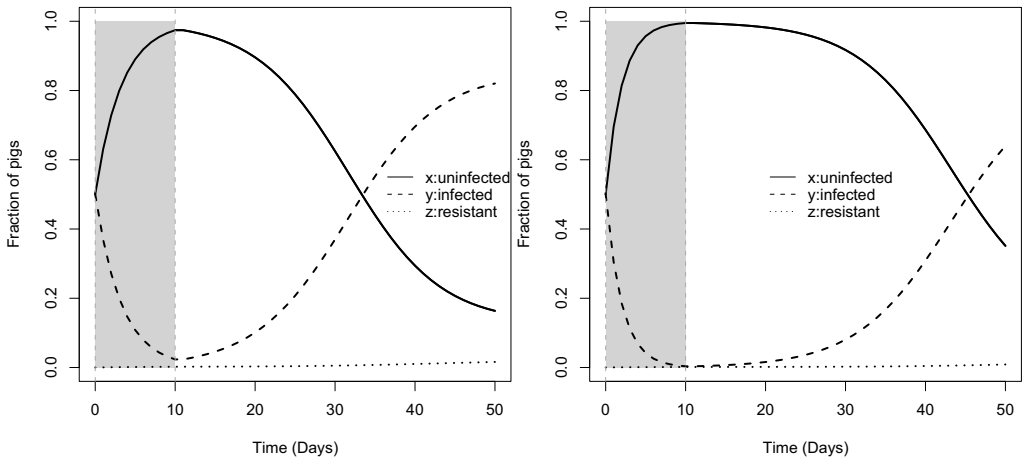
Fig. 4. Solution trajectories for system (3) with initial proportions : $x_0 = 0.5$, $y_0 = 0.5$ and $z_0 = 0$ and selected parameter values: $\beta_1 = 0.1$, $\beta_2 = 0.01$, $\alpha = 0.001$, $\gamma_1 = 0.04$, $\gamma_2 = 0.04$, *and* $\phi = 0.001$. The shaded region represents the treatment window. With 50% of pigs carrying drug-sensitive bacteria prior to treatment, treating at a daily rate of 0.3 led to a higher proportion of infected pigs as compared to that when treating at a daily rate of 0.5 prior to transport to slaughter. During treatment, in (a) $R_i = 0$ and $R_r = 0.28$ after treatment $0.34 \leq R_i \leq 2.17$ and $R_r = 0.10$. In (b) $R_i = 0$ and $R_r = 0.28$ during treatment whereas after treatment $0.22 \leq R_i \leq 2.02$ and $R_r = 0.10$. No pigs were found to carry resistant bacteria.

3.3.2 No pre-existing mutants: Episodic treatment

When animals are prescribed first-line treatment, the prevalence of infection is expected to decrease. However, due to the emergence of drug resistance, the infection may persist and alternative therapy (new drug or increase in dosage of first line treatment) may be employed. An episodic treatment, starting with a treatment with recovery rate 0.3 followed by 0.5 was evaluated for different values of the threshold parameters. In general, there is no standard waiting time between the first-line treatment and the second line treatment. This is decided upon by the veterinarians based on their observations of the health status of the infected animals and on experience. A waiting time of up to 15 days was used in the simulations. Assuming that 50% of the total pig population was infected with drug-sensitive bacteria and transmission parameters chosen such that $R_i < 1$ and $R_r < 1$, the infection was found to persist after

Accepted manuscript

ACCEPTED MANUSCRIPT



(a) $\tau = 0.3$ with treatment effect $\eta \leq 0.3$ (b) $\tau = 0.5$ with treatment effect $\eta \leq 0.5$

Fig. 5. The initial proportions were $x_0 = 0.5$, $y_0 = 0.5$ and $z_0 = 0$ and the selected parameter values were : $\beta_1 = 0.2,\ \beta_2 = 0.08,\ \alpha = 0.04,\ \gamma_1 = 0.02,\ \gamma_2 = 0.02, \phi = 0.001$ *and* $R_r > 1$. The shaded region represents the treatment window. Almost 3% of the pig population carried resistant bacteria at a recovery rate of 0.3 compared to almost 2% at a recovery rate of 0.5 prior to transport to slaughter. During treatment, in (a) $R_i = 0$ and $R_r = 6$ whereas after treatment $0.73 \leq R_i \leq 7.66$ and $R_r = 2.5$. In (b),during treatment $R_i = 0$ and $R_r = 6$ whereas after treatment $0.45 \leq R_i \leq 6.76$ and $R_r = 1.75$.

.

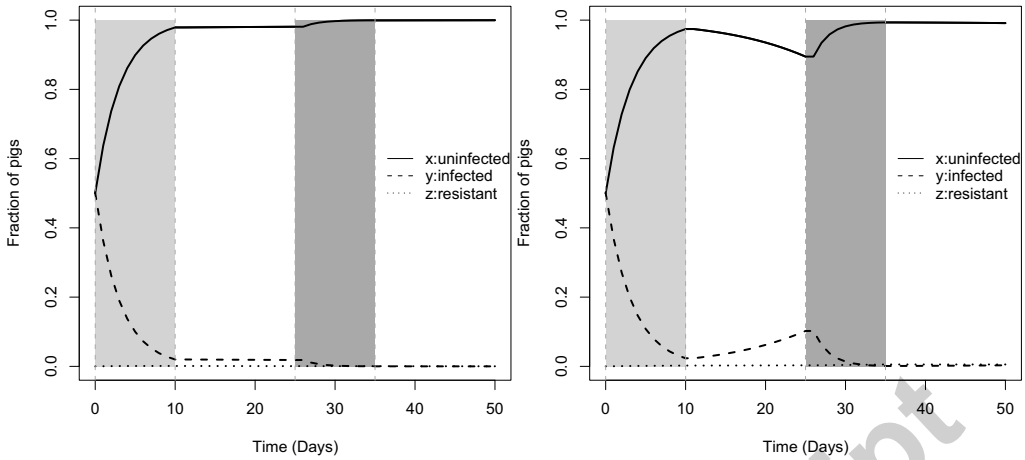
the first–line treatment (about 2% on day 25) with recovery rate 0.3. Following the second–line treatment that started at day 25 with a recovery rate of 0.5, the infection died out by day 30 (Fig. 6a). The situation changed when the parameters were such that $R_r > 1$. On day 25, about 10% of the pig population was still harboring drug-sensitive bacteria (Fig. 6b). On day 35 when treatment stopped, the fraction of infected pigs reduced to 0. At the end of the study period, only about 0.5% of the pigs were infected with drug-resistant bacteria.

Accepted manuscript

3.3.3 Pre-existing antimicrobial resistant mutants: First-line and episodic treatments

Given that the pig micro-flora is a huge reservoir for antimicrobial resistant genes, it is very likely that prior to treatment, resistant genes are already

ACCEPTED MANUSCRIPT



(a) $\tau = 0.3$ followed by $\tau = 0.5$, $R_r < 1$ (b) $\tau = 0.3$ followed by $\tau = 0.5$, $R_r > 1$

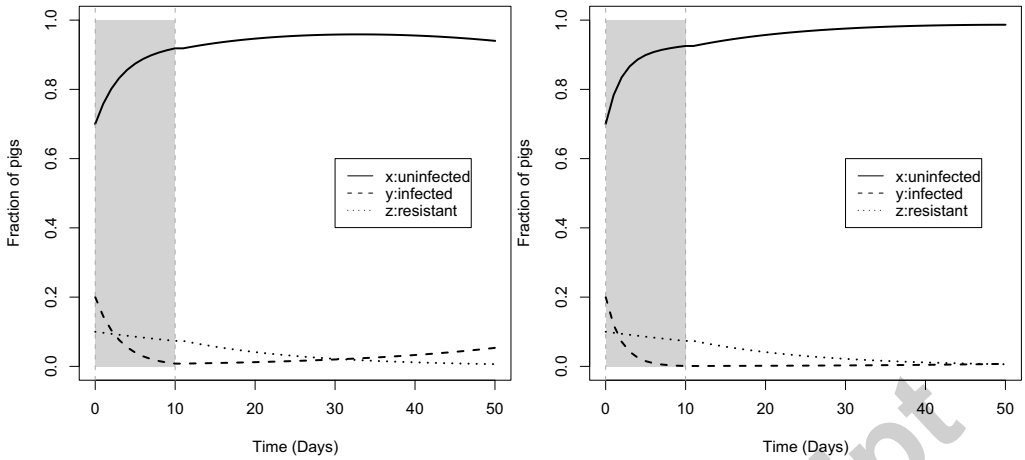
Fig. 6. Episodic treatment effect with no pre-existing antimicrobial resistant mutants. Initial proportions were $x_0 = 0.5$, $y_0 = 0.5$, $z_0 = 0$ and the selected parameter values were for (a): $\beta_1 = 0.1$, $\beta_2 = 0.01$, $\alpha = 0.01$, $\gamma_1 = 0.04$, $\gamma_2 = 0.04$, *and* $\phi = 0.001$ and for (b): $\beta_1 = 0.2$, $\beta_2 = 0.08$, $\alpha = 0.04$, $\gamma_1 = 0.02$, $\gamma_2 = 0.02$, *and* $\phi = 0.001$. The shaded regions represent the treatment windows (light gray=first-line treatment and dark gray=second-line treatment). In (a), $R_i = 0$ and $R_r = 0.28$ during the first line treatment whereas $0.34 < R_i < 0.96$ and $R_r = 0.12$ post first-line treatment. During the second-line treatment, $R_i = 0$ and $R_r = 0.28$ whereas $0.21 < R_i < 0.68$ and $R_r = 0.12$ post second-line treatment. In (b) $R_i = 0$ and $R_r = 6$ during the first-line treatment whereas $R_i = 0$ and $R_r = 1.8$ post first-line treatment. During the second-line treatment, $R_i = 0$ and $R_r = 6$ whereas $0.44 < R_i < 1.57$ and $R_r = 1.8$ post second-line treatment.

present in the pig population in the herd. With 10% of the pig population harboring resistant strains prior to treatment, administration of first-line treatment cleared out the resistant strains and some of the drug-sensitive strains (about 15%) at a recovery rate of 0.3 at the end of the study period (Fig. 7a). On the other hand, as shown on Fig. 7b, at a recovery rate of 0.5, almost all drug-sensitive and all drug-resistant strains were cleared out of the herd at the end of the study period. The threshold parameters R_i and R_r were all less than 1.

Next, a scenario was considered in which the threshold parameter $R_r > 1$ and

Accepted manuscript

ACCEPTED MANUSCRIPT



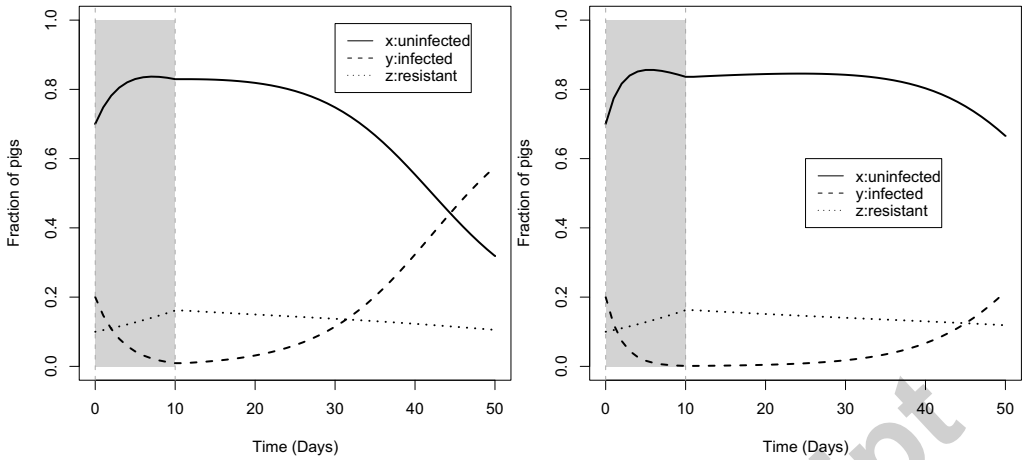
(a) $\tau = 0.3$ with treatment effect $\eta \leq 0.3$ (b) $\tau = 0.5$ with treatment effect $\eta \leq 0.5$

Fig. 7. Solution trajectories for a finisher herd with pre-existing antimicrobial resistant mutants. $x_0 = 0.7$, $y_0 = 0.2$, $z_0 = 0.1$ and the selected parameter values were: $\beta_1 = 0.1$, $\beta_2 = 0.01$, $\alpha = 0.001$, $\gamma_1 = 0.04$, $\gamma_2 = 0.04$, *and* $\phi = 0.001$. The shaded region represents the treatment window. During treatment, in (a) $R_i = 0$ and $R_r = 0.28$ whereas after treatment $0.34 \leq R_i \leq 2.17$ and $R_r = 0.09$. In (b), during treatment $R_i = 0$ and $R_r = 0.28$ whereas after treatment $0.22 \leq R_i \leq 2.02$ and $R_r = 0.09$

the values of R_i varied between 0 and 1. During treatment, the proportion of infected pigs was observed to decrease and those with resistant bacteria was observed to increase. Immediately after treatment stopped, the proportion of infected pigs was observed to increase steeply whereas those with resistant strains decreased only slowly (Fig. 8a and 8b). The increase in the proportion of pigs infected with drug-sensitive bacteria was steeper with a recovery rate of 0.3 as compared to that at a recovery rate of 0.5. Forty days post treatment, there were more infected pigs for treatment with recovery rate of 0.3 (about 58%) as compared to that of a recovery rate of 0.5 (about 22%). However, the proportion of pigs infected with drug-resistant bacteria at the end of the study period was almost the same for a treatment with recovery rate 0.5 (about 12%) as compared to that with a recovery rate of 0.3 (about 11%).

Accepted manuscript

ACCEPTED MANUSCRIPT



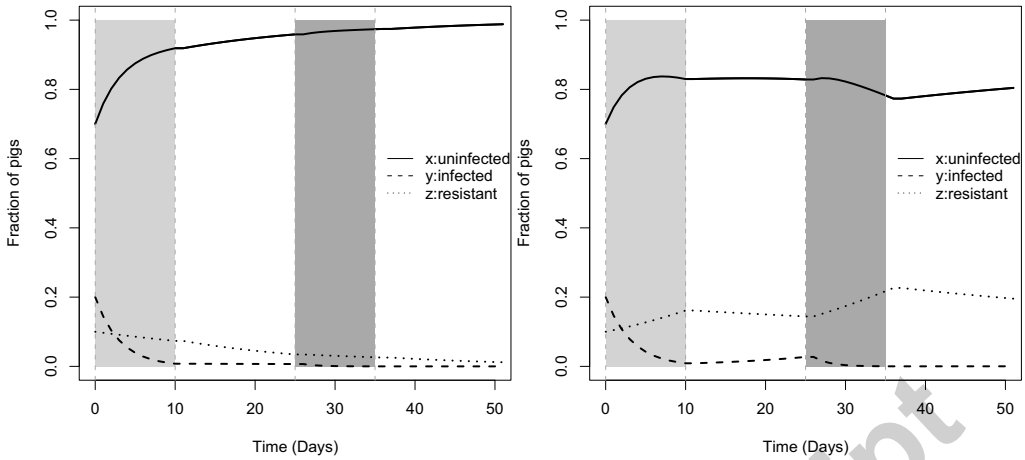
(a) $\tau = 0.3$ with treatment effect $\eta \leq 0.3$ (b) $\tau = 0.5$ with treatment effect $\eta \leq 0.5$

Fig. 8. Solution trajectories for a finisher herd with pre-existing antimicrobial resistant mutants. Initial proportions were $x_0 = 0.7$, $y_0 = 0.2$, $z_0 = 0.1$ and the selected parameter values were: $\beta_1 = 0.2$, $\beta_2 = 0.08$, $\alpha = 0.04$, $\gamma_1 = 0.02$, $\gamma_2 = 0.02$, *and* $\phi = 0.001$. The shaded region represents the treatment window. During treatment, in (a) $0.62 \leq R_i \leq 0.72$ and $R_r = 6$ after treatment $0.73 \leq R_i \leq 7.66$ and $R_r = 1.4$. In (b) $0.38 \leq R_i \leq 0.45$ and $R_r = 6$ during treatment whereas after treatment $0.45 \leq R_i \leq 6.76$ and $R_r = 1.4$

Finally, in the next scenario, the effects of an episodic treatment were tested where 10% of the pig population carried resistant bacteria and 20% carried drug-sensitive bacteria prior to treatment. Choosing transmission parameters such that $R_r < 1$ and $R_i < 1$ through out the treatment duration, the infection was cleared out prior to transport to slaughter (Fig. 9a). However, 1% of the total pig population was still infected with drug-resistant bacteria at the end of the study period. Increasing the threshold parameter such that $R_r > 1$, the proportion of pigs infected with drug-sensitive bacteria was reduced to zero at the end of the study period. However, prior to transport to slaughter about 22% of the pigs were still infected with drug-resistant bacteria (Fig. 9b).

Accepted manuscript

ACCEPTED MANUSCRIPT



(a) $\tau = 0.3$ with treatment effect $\eta \leq 0.3$ (b) $\tau = 0.5$ with treatment effect $\eta \leq 0.5$

Fig. 9. Episodic treatment effect for different initial proportion of infected pigs. $x_0 = 0.5$, $y_0 = 0.5$, $z_0 = 0$ and the selected parameter values are: $\beta_1 = 0.2$, $\beta_2 = 0.08$, $\alpha = 0.04$, $\gamma_1 = 0.02$, $\gamma_2 = 0.02$, *and* $\phi = 0.001$. The shaded region represents the treatment window. In (a), $0.29 < R_i < 0.34$ and $R_r = 0.28$ during the first line treatment whereas $0.34 < R_i < 0.97$ and $R_r = 0.11$ post first-line treatment. During the second-line treatment, $0.18 < R_i < 0.21$ and $R_r = 0.28$ whereas $0.21 < R_i < 0.68$ and $R_r = 0.11$ post second-line treatment. In (b) $0.62 < R_i < 0.72$ and $R_r = 6$ during the first-line treatment whereas $0.73 < R_i < 2.40$ and $R_r = 1.4$ post first-line treatment. During the second-line treatment, $0.37 < R_i < 0.43$ and $R_r = 6$ whereas $0.44 < R_i < 1.57$ and $R_r = 1.4$ post second-line treatment.

3.3.4 Uncertainty analysis

Uncertainty analysis was performed to investigate the uncertainties in the choice parameter values for the simulations and also to control for their influence on the outcome variable namely, the proportion of pigs infected with drug-resistant bacteria prior to transport to slaughter. Parameter values were chosen to reflect thresholds for the occurrence of an epidemic and the persistence of antimicrobial resistance in the herd. Little information was available about the distribution of the transmission parameters used in our simulations. Therefore, the simulation results of Fig. 8a were used with parameter values sampled from the Triangular distribution (Table 2). The mode of the trian-

Accepted manuscript

ACCEPTED MANUSCRIPT

gular distribution in each setting was the assumed value of the parameter estimate in Fig. 8a and the maximum and minimum values were arbitrarily chosen. We generated 10^3 samples from the assumed parameter distributions and used these to estimate the variability around the predicted proportion of pigs with resistant bacteria.

Table 2
Specification of minimum, mode and maximum of the triangular distribution (Tr.) characterizing each parameter in Fig. 8a

Parameter	**Label**	**Min**	**Mode**	**Max**	**Dist.**
Transmission rate (x*y)	β_1	*0.11*	*0.2*	*0.35*	*Tr.*
Transmission rate (x*z)	β_2	*0.05*	*0.08*	*0.12*	*Tr.*
Transmission rate (x*z)	β_3^*	*0.02*	*0.05*	*0.09*	*Tr.*
Transmission rate (y*z)	α_1	*0.01*	*0.04*	*0.06*	*Tr.*
Transmission rate (y*z)	α_2^*	*0.005*	*0.02*	*0.05*	*Tr.*
Sensitive clear-out rate	γ_1	*0.005*	*0.02*	*0.05*	*Tr.*
Resistance clear-out rate	γ_2	*0.005*	*0.02*	*0.05*	*Tr.*
Resistance clear-out rate	γ_3^*	*0.01*	*0.05*	*0.12*	*Tr.*
Mutation rate	ϕ	*0.0006*	*0.001*	*0.0017*	*Tr.*

*Dist.:= Distribution; * value of parameter after treatment stops*

Fig. 10 shows the solution trajectories of system (3) with 95% confidence bands. The estimated proportion of pigs that were uninfected, harbored drug-sensitive or drug-resistant bacteria prior to transport to slaughter were about 0.33 (95% C.I.=(0.26,0.41)), 0.55 (95% CI=(0.45,0.65)) and 0.12 (95% C.I.=(0.10,0.13)) respectively.

3.3.5 Sensitivity analysis

A sensitivity analysis was conducted to determine the relative contribution of each parameter in determining the proportion of pigs carrying drug-resistant

Accepted manuscript

ACCEPTED MANUSCRIPT

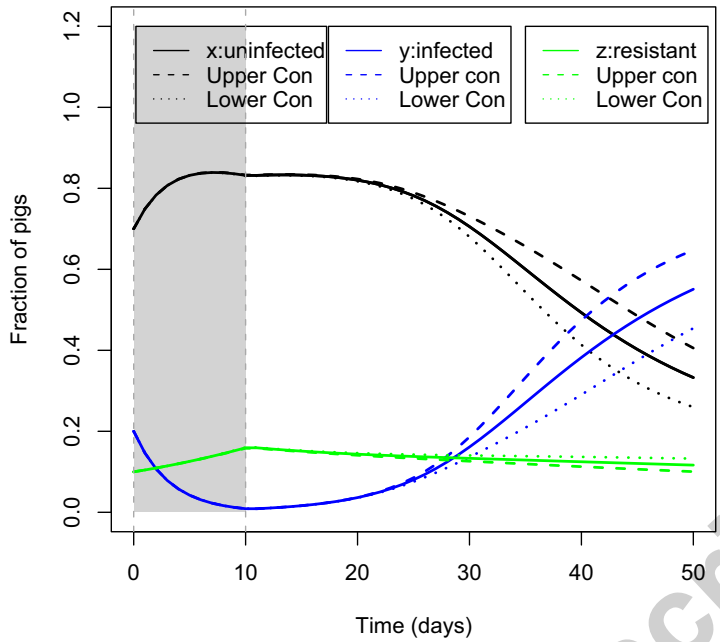


Fig. 10. Uncertainty analysis. Solution trajectories of system (3) with 95% confidence bands

bacteria (P_r). This was done by calculating the partial rank correlation coefficients (PRCC). The magnitude of the PRCC indicates the importance of the uncertainty in estimating each parameter as they independently contribute to the imprecision in predicating P_r (Blower and Dowlatabadi, 1994; Sanchez and Blower, 1997; Ivanek et al., 2004; Rong et al., 2007;). The PRCCs were estimated in R using the 'sensitivity' package. The estimated PRCCs are as shown on Table 3 in descending order of magnitude. PRCCs $> |0.5|$ indicate strong correlations between the model parameter and P_r.

The parameters β_2, β_3, γ_2 and γ_3 were found to exhibit high correlations with P_r. This corresponds to a high level of statistical influence these parameters have on P_r due to their own estimation uncertainty. An increase in the value of β_2 or β_3 will lead to a corresponding increase in the estimated P_r whereas an increase in the values of γ_2 or γ_3 will lead to a corresponding decrease in the value of P_r (Table 3).

Accepted manuscript

ACCEPTED MANUSCRIPT

Table 3
Partial rank correlation coefficients (PRCCs) between each model parameter and the estimated proportion of pigs carrying resistant bacteria (P_r) at the end the of the finishing period using 10^3 replications.

Input parameter	PRCC	95% Confidence interval
γ_3	-0.975	(-0.979,-0.973)
β_3	0.900	(0.886, 0.915)
β_2	0.817	(0.798, 0.846)
γ_2	-0.746	(-0.782,-0.716)
α_2	0.204	(0.130,0.275)
β_1	-0.154	(-0.216,-0.084)
γ_1	0.058	(0.002,0.111)
α_1	0.016	(-0.040,0.082)
ϕ	0.010	(-0.062,0.070)

4 Discussion

In this study, a mathematical model was established for understanding the dynamics of drug-sensitive and drug-resistant bacteria in a finisher pig herd and for predicting the proportion of pigs with drug-resistant bacteria prior to transport to slaughter. The model is similar in formulation to those of Austin et al. (1997) and Kavanagh et al. (2005). However the models by Austin et al. (1997) and Kavanagh et al. (2005) assumed that the treatment effect was constant whereas in our model, a more realistic time-varying treatment effect was assumed. Our model can be adapted to study the transmission dynamics of a commensal bacteria such as *E. coli* as well as a zoonotic bacteria such as *Salmonella* in animals following the models proposed by Austin et al. (1997) and Kavanagh et al. (2005) respectively.

Our model predicted that the higher the initial proportion of infected pigs, the greater the likelihood that resistance will emerge and the higher the proportion

Accepted manuscript

ACCEPTED MANUSCRIPT

of pigs still carrying drug-sensitive bacteria prior to transport to slaughter. In addition, if a fraction of the pig population already carries drug-resistant bacteria prior to treatment administration, the resistant strains will die out if the magnitude of the transmission parameters are small ($R_r < 1$) and may persist otherwise.

For the first-line treatment, it was observed that when no pigs carrying drug-resistant bacteria were present in the herd, increasing the recovery rate led to an increase in the number of days post treatment at which the infection starts to re-emerge in the pig population. This implies that prior to transport to slaughter, the prevalence of the infection will be low. This is desirable because an infection with a low prevalence will have a lower chance of escalating. Also, at the same recovery rate, treatment was more effective for a herd with a smaller fraction of infected pigs. This is a suggestion that in the event of an infection in the pig herd, a drug with a high recovery rate should be employed. Also, it was derived that to arrive at an infection-free state, where no pigs infected with drug-sensitive or drug-resistant bacteria were present in the herd prior to transport to slaughter, $R_i < 1$ and $R_r < 1$. This can also be interpreted to mean that if the proportion of drug-sensitive and drug-resistant pigs are small, then a combination of appropriate treatment and host's defense will clear off bacteria from the pigs and drive the system to the infection-free steady state. Similar conclusions have been obtained in other studies (van den Driessche and Watmough, 2002). However, the effect of the host's immune system, which has been shown to play a key role in treatment success (Alavez-Ramirez et al., 2007) was not included in our analysis for the sake of model simplification.

Several simplifying assumptions were made during the modeling process. Pro-

Accepted manuscript

ACCEPTED MANUSCRIPT

vided that the death rate was the same for all pigs in the different infection states, the assumption of zero mortality is considered to have little effect on the transmission dynamics of resistant bacteria in a pig herd. However, if different mortality rates are associated with pigs in the different infection states, the assumption of zero mortality might lead to incorrect estimates for the proportion of pigs in the different infection states in the farm. For such infections, the mortality rates should be included in the model. By ignoring the details of the pharmacokinetics and pharmacodynamics, and considering only the treatment effect, it was feasible to model the effects of different drugs. This offered a platform for combining several therapies. However, this assumption might have caused the model to fail to capture the true treatment effect of the drugs under consideration. It is the hope that, tailored experiments be conducted in the future to support this assumption. In addition, the bi-exponential model was used to describe the dynamics of the treatment effect since it usually provides a better fit to compared to other models such as the mono-exponential and poly-exponential models (Davies et al., 2006). Future studies in which the fit of several models are compared are warranted. It was assumed that drug-resistant pigs recovered spontaneously faster than drug-sensitive pigs although they have a lower transmission rate. The energy burden associated with mutations and plasmid transfer thus rendered drug-resistant bacteria less competitive (Austin et al., 1997; Banhoeffer et al., 1997; Lenski, 1998). However, it has been argued that due to the fast adaption of drug-resistant bacteria ushered by compensatory mutations, the competition ties up (Levin et al., 2000). The predicted proportion of pigs with drug-resistant prior to transport to slaughter might therefore have been under estimated. The numerical simulations presented in this study, were based on chosen parameters (not strictly from experimental studies or expert opinion). These were selected

Accepted manuscript

ACCEPTED MANUSCRIPT

so that the threshold parameters (R_i and R_r) could reflect the steady state solutions of the system. The parameter values might therefore not give a true reflection of actual dynamics in the herd. This was however due to the fact that studies of such nature have not been published in the literature. This highlights the need for designed experiments to estimate model parameters. Finally, the assumption of random contact amongst animals might have over-estimated the predicted proportion of pig with drug-resistant bacteria. This is the case, provided pigs were grouped in pens of very small sizes. However, the model presented here considers a large herd where pen partitioning was not considered.

The mathematical model we developed was based on the underlying assumption that pigs in the drug-sensitive class were infected with predominantly drug-sensitive bacteria whereas those in the drug-resistant class were assumed to be infected with predominantly drug-resistant bacteria. This clear-cut distinction was for the sake of simplicity because pigs are usually infected with both drug-sensitive and drug-resistant bacteria at the same time. This implies that the infection status of the animals may change rapidly if one of the strains out-competes the other. Our model did not take account of such state transitions and this might have led to an under/over-estimation of the proportion of pigs in each of the infection states. An integrated or nested model where the effects of antimicrobial usage on the within and between host dynamics are linked can be used to resolve the aforementioned concern (McKenzie and Bossert, 2005; Gilchrist and Coombs, 2006)

Sensitivity analysis yielded that two parameters had a huge impact on the predicted proportion of pigs with drug-resistant bacteria prior to transport to slaughter: a) the transmission coefficient between susceptible pigs and those

Accepted manuscript

ACCEPTED MANUSCRIPT

with drug-resistant bacteria during treatment (β_2) and after treatment stops (β_3), and b) the spontaneous clear-out rate of drug-resistant bacteria during treatment (γ_2) and after removal of antimicrobial pressure (γ_3). Therefore to reduce the proportion of pigs carrying drug-resistant bacteria prior to transport to slaughter, control measures should be geared towards reducing the magnitudes of the former and increasing those of the later. This is already being achieved in many animal production systems in the world today. For example, in Denmark pigs are housed in pens partitioned such that the likelihood of pen to pen transmission are reduced (Anonymous, 2006). Also, most farms are equipped with hospital pens where sick animals can be treated in isolation and thus reducing the transmission of the infection.

It has previously been observed that so long as antimicrobial resistant mutations are present in host, drug resistance will always emerge once a new drug is introduced (Austin et al., 1997; Rong et al., 2007). In our study, it was demonstrated that this depends in turn on the recovery rate and on the magnitude of the transmission parameters. Using first-line treatment, resistance was observed to die out when the transmission parameters were such that $R_r < 1$. However, pigs with resistant bacteria persisted in the herd when $R_r > 1$. When a second-line treatment was used, the proportion of pigs with drug-resistant bacteria was completely eradicated from the pig herd if transmission parameters were such that $R_r < 1$. Pigs with drug-resistant bacteria however persisted in the farm prior to transport to slaughter when $R_r > 1$.

The proposed model was deterministic in nature. The advantage of modeling in this platform is the simplicity of the analyzes. However, these models described the transmission of resistant bacteria in the herd under the assumption of the mass action principle. Because this in turn relies on the law of large

Accepted manuscript

ACCEPTED MANUSCRIPT

numbers, the model can best be used for large herds (Anderson and Britton, 2000). Otherwise, stochastic models could be more suitable for example if modeling smaller units in the herd such as pens. Also, stochastic models can be very useful for estimating model parameters since the transmission process is modeled in terms of probabilities. Even though stochastic models are best for estimating uncertainties around parameters or target quantities, it was possible to do same using our deterministic models.

In spite of the limitations of the proposed modeling framework, it was demonstrated that the persistence of pigs with drug-resistant bacteria in a finisher pig herd depends on the magnitude of the transmission parameters and whether or not antimicrobial resistant bacteria were already present in the pig population prior to transport to slaughter. In addition, it was shown that two parameters influence the estimated proportion of pigs carrying drug-resistant bacteria prior to transport to slaughter a) the transmission coefficient between susceptible pigs and those with drug-resistant bacteria during treatment (β_2) and after treatment stops (β_3), and b) the spontaneous clear-out rate of drug-resistant bacteria during treatment (γ_2) and after removal of antimicrobial pressure (γ_3). Any control measure that will reduce the transmission rate or increase the spontaneous clear-out rate will definitely reduce the proportion of pigs with drug-resistant bacteria prior to transport to slaughter.

Accepted manuscript

Acknowledgements

This research project was supported by a grant from the EU Marie Curie Programme (MEST-CT-2004-007819).

ACCEPTED MANUSCRIPT

References

Alavez-Ramirez, J., Castellanos, J. R., Esteva, L., Flores, J. A., Fuentes-Allen, J. L., Garcia-Ramos, G., Gomez, G., Lopez-Estrada, J., 2007. Within-host population dynamics of antibiotic-resistant M. tuberculosis . Math. Med. and Biol. 24 (1), 35–56.

Altekruse, S., Stern, N., Fields, P., Swerdlow, D., 1999. *Campylobacter* jejuni an emerging foodborne pathogen. Emerg. infect. Dis. 5, 28–35.

Anderson, H., Britton, T., 2000. Stochastic epidemic models and their statistical analyis. Springer Lecture Notes in Statistics. Vol. 151. Springer-Verlag, New York.

Angulo, F. J., Nargund, V. N., Chiller, T. C., 2004. Evidence of an Association Between Use of Anti-microbial Agents in Food Animals and Anti-microbial Resistance Among Bacteria Isolated from Humans and the Human Health Consequences of Such Resistance. Vet. Med. B (5), 374379.

Anonymous, 2006. Danish pig production: Annual report. Danish Bacon and Meat Council.

Anonymous, 2007. The Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (A combined reference of 11 DANMAP reports, 1997-2007). www.danmap.org.

Asai, T., Akemi, K., Kazuki, H., Kanoko, I., Toshio, T., Yutaka, T., 2000. Correlation between the usage volume and veterinary therapeutic antimicrobials and resistance in *Escherichia Coli* isolated from the feces of food-producing animals in Japan. Genetics 58, 369–372.

Austin, D. J., Kakeshashi, M., Anderson, M., 1997. The transmission dynamics of antibiotic-resistant bacteria: the relationship between resistance in commensal organisms and antibiotic consumption. Proc. R. Soc. Lond. B. 264, 1629–1638.

Accepted manuscript

ACCEPTED MANUSCRIPT

Banhoeffer, S., Lipsitch, M., Levin, B. R., 1997. Evaluating tretament protocols to prevent antibiotic resistance. . Proc. Natl. Acad. Sci. USA 94 (3), 12106–12111.

Barbosa, T. M., Levy, S. B., 2000. The impact of antibiotic use on resistance development and persistence. Drug Resist. Update. 3, 303–311.

Bibbal, D., Dupouy, V., Ferre, J. P., Toutain, P. L., Fayet, O., Prere, M. F., Bousquet-Melou, A., 2007. Impact of three ampicillin dosage regimens on selection of ampicillin resistance in enterobacteriaceae and excretion of blatem genes in swine feces. Appl. Environ. Microbiol. 73 (15), 4785–4790.

Blower, S. M., Dowlatabadi, H., 1994. Sensitivity and Uncertainty Analysis of Complex Models of Disease Transmission: An HIV Model, as an Example. Int. stat. rev 62 (2), 229–243.

Davies, G. R., Brindle, R., Khoo, S. H., Aarons, L. J., 2006. Use of Nonlinear Mixed-Effects Analysis for Improved Precision of Early Pharmacodynamic Measures in Tuberculosis Treatment. Antimicrob. Agents Chemother. 50 (9), 3154–3156.

Delsol, A. A., Randall, L., Cooles, S., Woodward, M. J., Sunderland, J., Roe, J., 2005. Effect of the growth promoter avilamycin on emergence and persistence of antimicrobial resistance in enteric bacteria in the pig. J. Appl. Microbiol. 1 (1), 4–27.

Edelstein-Keshet, L., 2005. Mathematical models in biology. Society for Industrial and Applied Mathematics, Philadelphia.

Engberg, J., Keelan, M., Gerner-Smidt, P., Taylor, D. E., 2006. Antimicrobial resistance in *Campylobacter*. In: Antimicrobial resistance in bacteria of animal origin. ASM Press, Washington DC, pp. 269–291.

Esteva, L., Vargas, C., 1998. Analysis of a dengue disease transmission model. Math. Biosci. 150, 131–151.

Accepted manuscript

ACCEPTED MANUSCRIPT

Gilchrist, M. A., Coombs, D., 2006. Evolution of virulence: Interdependence, constraints, and selection using nested models. Theor. Popul. Biol. 69, 145–153.

Greenblatt, D., von Moltke, L. L., Harmatz, J. S., Shader, R. I., 2002. Pharmacokinetics, pharmacodynamics, and drug disposition. In: Neuropsychopharmacology: the Fifth Generation of Progress, eds Davis KL, Charney D, Coyle JT, Nemeroff C. Philadelphia. Lippincott, Williams and Wilkins, pp. 507–524.

Hethcote, H. W., 2000. The mathematics of infectious diseases. Society for Industrial and Applied Mathematics 42 (4), 599–653.

Huang, Y., Rosenkranz, S. L., Wu, H., 2003. Modeling HIV dynamics and antiviral response with consideration of time-varying drug exposures, adherence and phenotypic sensitivity. Math. Biosci. 184, 165–186.

Ivanek, R., Snary, E. L., Cook, A. J. C., Grohn, Y., 2004. A mathemctaical model for the transmission of *Salmonella typhimirium* within a grower-finisher pig herd in Great Britain. J. Food Prot. 67 (11), 2403–2409.

Jensen, V. F., Jakobsen, L., Emborg, H.-D., Seyfarth, A. M., Hammerum, A. M., 2006. Correlation between apramycin and gentamicin use in pigs and an increasing reservoir of gentamicin-resistant *Escherichia coli*. J. Antimicrob. Chemother. 1 (1), 4–27.

Kavanagh, K., Kelly, L., Gettinby, G., Snary, E., 2005. Emergence of antibiotic resistant *Salmonella Typhimurium*: The effect of antibiotic therapy on a single pen of grower-finisher pigs. In: Proceedings of the SVEPM International Conference. Scotland.

Korobeinikov, A., 2006. Lyapunov functions and global stability for SIR and SIRS epidemiological models with non-linear transmission. Bull. Math. Biol. 68 (3), 615–626.

Accepted manuscript

ACCEPTED MANUSCRIPT

LaSalle, J. P., 1976. The Stability of Dynamical Systems. SIAM 1.

Lenski, R. E., 1998. Bacterial evolution and the cost of antibiotic resistance . Internatl. Microbiol. 1, 265–270.

Levin, B. R., Perrot, V., Walker, N., 2000. Compensatory Mutations, Antibiotic Resistance and the Population Genetics of Adaptive Evolution in Bacteria. Jpn. J. Infect. Dis. 154, 985–997.

Levin, S. A., Andreason, V., 1999. Disease transmission dynamics ad the evolution of antibiotic resistance in hospitals and communal settings. Proc. Natl. Acad. Sci. USA 96, 800–801.

Lipsitch, M., Samore, M. H., 2002. Antimicrobial use and antimicrobial resistance: A population perspective. Emerg. Infect. Dis. 8 (4), 347–354.

McDermott, P., 2006. Antimicrobial resistance in Nontyphoidal *Salmonellae*. In: Antimicrobial resistance in bacteria of animal origin. ASM Press, Washington DC, pp. 293–314.

McGowan, J. E., 2001. Economic impact of antimicrobial resistance. Emerg. Infect. Dis. 7 (2), 286–292.

McKenzie, F. E., Bossert, W. H., 2005. An integrated model of *Plasmodium* falciparium dynamics. J. Theor. Biol. 232, 411–426.

Rong, L., Feng, Z., Perelson, A. S., 2007. Emergence of HIV-1 Drug Resistance During Antiretroviral Treatment. Bull. Math. Biol. 69, 2027–2060.

Sanchez, M. A., Blower, A. M., 1997. Uncertainty and sensitivity analysis of the basic reproduction rate . Am. J. Epidemiol. 145 (12), 1127–1137.

Smith, D. L., Harris, A. D., Johnson, J. A., Silbergeld, E. K., Morris, J. G., 2002. Animal antibiotic use has an early but important impact on the emergence of antibiotic resistance in human commensal bacteria. Proc. Natl. Acad. Sci. USA 99 (9), 6434–6439.

Teale, C. J., 2004. Antimicrobial resistance and the food chain. J. App. Mi-

Accepted manuscript

ACCEPTED MANUSCRIPT

crobiol. 92 (1), 85–89.

van den Driessche, P., Watmough, J., 2002. Reproduction numbers and subthreshold endemic equilibria for compartmental models of disease transmission . Math. Biosci. 180, 29–48.

White, D. J., McDermott, P. F., 2006. Emergence and Transfer of Antibacterial Resistance. J. Dairy Sci. 1 (84), 151–155.

Wiuff, C., Lykkesfeldt, J., S. O., F.M., A., 2003. The effects of oral and intramuscular administration and dose escalation of enrofloxacin on the selection of quinolone resistance among *Salmonella* and coliforms in pigs. Res. Vet. Sci. 75 (3), 185–193.

Wong, D., Colburn, W. A., Gibaldi, M., 1979. Fitting Concentration-Time Data to Biexponential Equations. J. Pharmacokinet. Biopharm. 7 (1), 97–100.

Accepted manuscript

Appendix

Stability of resistant-free steady state

For $E_1 = (x^+, y^+, 0)$ where $x^+ = \frac{1}{R_i}$, and $y^+ = 1 - \frac{1}{R_i}$ reduced to $E_{11} = (y^+, 0)$. The variational (stability) matrix of system (3) is given by:

$$\mathbf{J}(E_{11}) = \begin{pmatrix} m_{11} & m_{12} \\ \phi & m_{22} \end{pmatrix}$$

where $m_{11} = -\beta_1(\frac{R_i-1}{R_i})$, $m_{12} = -(\alpha+\beta_1)(\frac{R_i-1}{R_i})$, $m_{21} = \phi$, and

ACCEPTED MANUSCRIPT

$$m_{22} = (\beta_2 - \gamma) + (\tfrac{R_i - 1}{R_i})(\alpha - \beta_2)$$

The eigen values of $\mathbf{J}(E_{11})$ satisfy: $det(\mathbf{J}(E_{11}) - \lambda^{-}\mathbf{I}) = 0$, from which the following characteristic equation is obtained:

$$0 = \lambda^{-2} + A_1\lambda^{-} + A_2$$

where $A_1 = -m_{11} - m_{22}$ and $A_2 = m_{11}m_{22} - \phi m_{12}$

Solving this, we obtain that

$$\lambda_1^{-} = \frac{-A_1 + \sqrt{A_1^2 - 4A_2}}{2} \quad \text{and} \quad \lambda_2^{-} = \frac{-A_1 - \sqrt{A_1^2 - 4A_2}}{2}$$

These eigen values are all real and negative if $\sqrt{A_1^2 - 4A_2} > 0$, $A_1 > 0$ and $A_1 > \sqrt{A_1^2 - 4A_2}$. These conditions are all true only if $R_i > 1$, $R_r < 1$, and $\alpha < \beta_2$. It follows therefore that the eigen values are all real and negative from which it can be concluded that $E_1 = (x^+, y^+, 0)$ is locally asymptotically stable.

The global stability of the resistant free steady state is established in the following proposition:

Proposition 1.3: All solutions in the region Ω will approach the resistant-free equilibrium $E_1 = (x^+, y^+, 0)$ for $R_i \geq 1$ and $R_r \leq 1$ and $\alpha \leq \beta_2$.

Proof:

Consider the Lyapunov function: $V : \Omega \to \mathbb{R}$, $V = z$

which is well defined and continuous in Ω. The orbital derivative of V is

$$\dot{V} = \dot{z}$$

Accepted manuscript

ACCEPTED MANUSCRIPT

$$= \beta_2 xz + \alpha yz + \phi y - \gamma z$$

$$= z[\beta_2 x - \gamma] + \alpha yz + \phi y$$

Adding and subtracting αxz, and using the equation $\frac{(\alpha+\beta_2)}{\gamma} = R_r$ we have

$$\dot{V} = \gamma z[R_r x - 1] + \alpha yz - \alpha xz + \phi y$$

$$= \gamma z[R_r x - 1] + \alpha z[y - x] + \phi y$$

$$= \gamma z[R_r x - 1] + \alpha z[y - y^- + y^- - x] + \phi y$$

$$= \gamma z[R_r x - 1] + \alpha z[y - y^-] - \alpha z[x - y^-] + \phi y$$

$$< \gamma z[R_r x - 1] + \alpha z[y - y^-] + \phi y$$

At $E_2 = (x^-, y^-, 0)$, equation 2 of system (3) yields $\phi y^- = 0$ i.e $\phi = 0$ since $y^- > 0$ so,

$$\dot{V} < \gamma z[R_r x - 1] + \alpha z[y - y^-] \leq 0$$

$$\text{for } R_i \geq 1 \text{ and } R_r \leq 1 \text{ and } \alpha \leq \beta_2$$

If $R_i > 1$ and $R_r > 1 : \dot{V} = 0$ iff $z = 0$

If $R_r = R_i = 1 : \dot{V} = 0$ iff $x = x^-$ and $y = y^-$

It thus follows from the Lyapunov-Lasalle (LaSalle, 1976) theorem that E_1 is globally asymptotically stable in Ω and so every trajectory of system (3) approaches the resistant-free steady state for $R_i \geq 1$ and $R_r \leq 1$ and $\alpha \leq \beta_2$

Accepted manuscript

ACCEPTED MANUSCRIPT

Stability of resistant steady state

The resistant steady state $E_2 = (x^-, 0, z^-)$ where $x^- = \frac{\gamma}{\beta_2}$, and $z^- = 1 - \frac{\gamma}{\beta_2}$ is a state of the system where the pigs with drug-resistant bacteria are dominant in the farm. The stability of E_2 can be studied via the reduced system $E_{22} = (0, z^-)$. The variational (stability) matrix is given by:

$$\mathbf{J}(E_{22}) = \begin{pmatrix} m_{11} & 0 \\ m_{21} & m_{22} \end{pmatrix}$$

where $m_{11} = \beta_1[\frac{(R_i-1)}{R_i} - \frac{(\beta_2-\gamma)}{\beta_2}(1+\frac{\alpha}{\beta_1})]$, $m_{21} = (\beta_2 - \gamma)(\frac{\alpha}{\beta_2} - 1) + \phi$, and

$$m_{22} = (\gamma - \beta_2).$$

The eigen values of $\mathbf{J}(E_{22})$ satisfy: $det(\mathbf{J}(E_{22}) - \lambda^+\mathbf{I}) = 0$, from which the following characteristic equation is obtained:

$$0 = (m_{11} - \lambda^+)(m_{22} - \lambda^+)$$

For $E_{22} = (0, z^-)$ to be locally asymptotically stable, all eigen values of $\mathbf{J}(E_{22})$ must be negative.

$\lambda_1^+ = m_{11} < 0$ if $R_i < 1$ and $\beta_2 - \gamma > 0$. i.e. if $\beta_2 > \gamma$ or $\beta_2 + \alpha > \gamma$ for $\alpha > 0$. This is equivalent to saying that $R_r > 1$. It therefore follows that $\lambda_1^+ < 0$ iff $R_i < 1$ and $R_r > 1$. In addition, $\lambda_2^+ = m_{22} < 0$ if $\gamma - \beta_2 < 0$ which is equivalent to $R_r > 1$ following the previous explanations.

It can thus be concluded that E_{22} is locally asymptotically stable if and only

Accepted manuscript

ACCEPTED MANUSCRIPT

if $R_i < 1$ and $R_r > 1$ and unstable otherwise. When E_0 and E_2 both exists, and E_2 is stable, then E_0 will be unstable. This steady state reflects complete failure of drug to triumph over sensitive bacteria.

The global stability of this steady state is established in the following proposition:

Proposition 1.2: All solutions in the region Ω will approach the resistant equilibrium $E_2 = (x^-, 0, z^-)$ for $R_i \leq 1$ and $R_r \geq 1$.

Proof:

Consider the Lyapunov function: $V : \Omega \to \mathbb{R}$, $V = y$

which is well defined and continuous in Ω. The orbital derivative of V is

$$\begin{aligned}
\dot{V} &= \dot{y} \\
&= (\beta_1 xy - \alpha yz - (\tau + \gamma + \phi)y) \\
&= \beta_1 y[x - \tfrac{1}{R_i} - \tfrac{\alpha z}{\beta_1}] \\
&= \beta_1 y[\tfrac{x\beta_2 R_i - \beta_2}{\beta_2 R_i} - \tfrac{\alpha z}{\beta_1}] \\
&= y\beta_1[\tfrac{x\beta_2 R_i - (\alpha+\beta_2)}{\beta_2 R_i} + \tfrac{\alpha}{\beta_2 R_i} - \tfrac{\alpha z}{\beta_1}] \\
&= y\beta_1[\gamma(\tfrac{x}{\gamma} - \tfrac{R_r}{\beta_2 R_i}) + \tfrac{\beta_1(\alpha+\beta_2) - \alpha\beta_2 R_i z - \beta_1\beta_2}{\beta_1\beta_2 R_i}] \\
&= y\beta_1[(x - \tfrac{\gamma R_r}{\beta_2 R_i}) + \tfrac{\beta_1\gamma R_r - \alpha\beta_2 R_i z - \beta_1\beta_2}{\beta_1\beta_2 R_i}] \\
&= y\beta_1[(x - \tfrac{R_r}{R_i}x^+) + \tfrac{\beta_1\gamma R_r - \alpha\beta_2 R_i z - \beta_1\beta_2}{\beta_1\beta_2 R_i}]
\end{aligned}$$

At $E_1 = (x^+, 0, z^+)$, equation 2 of system (3) yields $\frac{1}{R_i} = x^+ - \frac{\alpha}{\beta_1}z^+$ so,

$\dot{V} = y\beta_1[(x - \frac{R_r}{R_i}x^+) + \frac{\gamma R_r}{\beta_2 R_i} - \frac{\alpha}{\beta_1}z - (x^+ - \frac{\alpha}{\beta_1}z^+)]$, using the fact that

Accepted manuscript

ACCEPTED MANUSCRIPT

$x^+ = \frac{\gamma}{\beta_2}$

$$= y\beta_1[(x - \frac{R_r}{R_i}x^+) + \frac{\gamma}{\beta_2}(\frac{R_r}{R_i} - 1) - \frac{\alpha}{\beta_1}(z^+ - z)] \leq 0$$

for $R_i \leq 1$ and $R_r \geq 1$

If $R_i > 1$ and $R_r > 1 : \dot{V} = 0$ iff $y = 0$

If $R_r = R_i = 1 : \dot{V} = 0$ iff $x = x^+$ and $z = z^+$

It thus follows from the Lyapunov-Lasalle (LaSalle, 1976)theorem that E_2 is globally asymptotically stable in Ω and so every trajectory of system (3) approaches the resistant steady state for $R_i \leq 1$ and $R_r \geq 1$.

Accepted manuscript