

Why, when and how should exposure be considered at the within-host scale? A modelling contribution to PRRSv infection

– Supplementary material –

Complete model description & calibration
Combined influence of within-host mechanisms and exposure on the viral load

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1. Complete model description

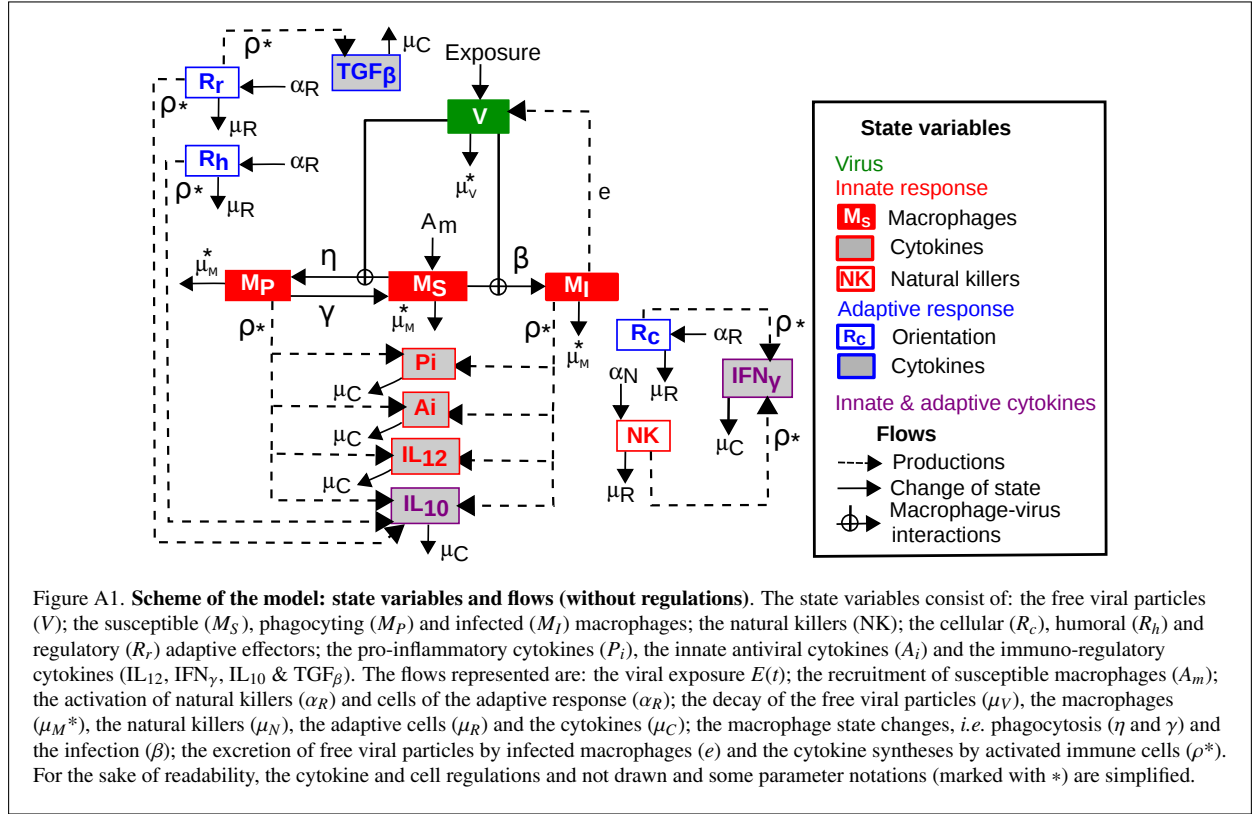
1.1. Model presentation

The model presented here is a simplified version of a model that we published previously [1]. Changes are highlighted *in italics* in the following description, which includes all equations and details our modelling assumptions.

The model aims at simulating the infection and immune dynamics induced by PRRSv in the lung. We chose a deterministic continuous-time dynamic framework. Our model is hence a set of 14 ordinary differential equations, which represent the evolution over time of the state variables: the free viral particles (V); the susceptible (M_S), phagocytosing (M_P) and infected (M_I) macrophages; the natural killers (NK); the cellular (R_c), humoral (R_h) and regulatory (R_r) adaptive effectors; the pro-inflammatory (P_i , grouping $IL_{1\beta}$, IL_6 and IL_8), innate antiviral (A_i , grouping IFN_α and TNF_α) and immuno-regulatory cytokines (IL_{12} , adaptive anti-viral: IFN_γ , immuno-modulatory: IL_{10} & TGF_β). The main processes that drive their evolution over time and that are integrated in the model are: the phagocytosis of the viral particles by the macrophages; the macrophage infection by the virus; the excretion of free viral particles by the infected macrophages; the recruitment and decay/migration of the macrophages; the activation and decay/migration of the other effectors; the cytokine productions by the immune cells and their decay; the cytokine regulations.

The functional diagram of the system appears in **Figure 1** (main text). Figure A1 gives a schematic representation of the model (without regulations). The cytokine regulations and syntheses represented in our model, as well as the related literature references are summarised in Table A1 and Table A2 respectively. The parameter descriptions and values are synthesised in Table A4.

We first present how the cytokine regulations are formalised in the model and then describe the dynamics of the various state variables: macrophages, free viral particles, natural killers, effectors of the adaptive response and cytokines. Finally, we present the model calibration: the method, based on the literature data exhibiting a strong variability (illustrated for the viral titer in Table A3), the parameter values in Table A4 and the comparison of model outputs between the previous and the present models in Figure A2.



1.2. Cytokine regulations

The processes driving the state variable dynamics are regulated by a complex cytokine feedback system (presented in **Figure 1**, main text). Some cytokines have opposite effects: for example, the antiviral cytokines up-regulate the phagocytosis and down-regulate the viral infection, whereas IL_{10} inhibits the phagocytosis and promotes the infection. Cytokines regulate the cellular functions through their recognition by specific receptors, inducing cascaded reactions within the cells. The higher the cytokine concentration, the stronger the effect. However, there is a limited number of cytokine receptors on the cell surface, so the effect saturates above a given cytokine concentration.

We formalised the cytokine regulations (up κ^+ and down κ^-) based on the Michaelis–Menten function of the cytokine concentration (C_i) [2, 3, 4] as follows:

$$\kappa^+(C_i) = \frac{v_m C_i}{k_m + C_i}, \quad \kappa^-(C_i) = \frac{k_m}{k_m + C_i},$$

where v_m denotes the saturation factor and k_m the half saturation constant.

Compared to [1], the down-regulation function κ^- is simplified.

A cytokine can have three possible effects on a given basic rate (r):

- activation: $r \kappa^+(C_i)$, the basic rate increases with the cytokine concentration from 0 to $r v_m$;
- amplification: $r [1 + \kappa^+(C_i)]$, the basic rate increases with the cytokine concentration from r to $r (1 + v_m)$;
- inhibition: $r \kappa^-(C_i)$, the basic rate decreases and tends to zero.

Regulations often involve several cytokines (C_i and C_j) which can act

- either independently: $\kappa^\pm(C_i + C_j)$;
- or in synergy:

- $\kappa^+(C_i C_j) = \frac{v_m C_i C_j}{k_m + C_i C_j}$ for an activation or an amplification,
- $\kappa^-(C_i C_j) = \frac{k_m^2}{k_m + C_i C_j}$ for an inhibition.

Compared to [1], effects involving multiple cytokines are simplified.

As very few studies estimate the regulation parameters (k_m and v_m) in the literature [4], we used the same parameter values for all cytokine regulations.

1.3. Macrophage dynamics

Macrophages phagocyte and destroy the virus, but they are also target cells for the virus. Activated macrophages (either phagocytosing or infected) present the viral antigen and activate the adaptive response. The macrophage activation also induces the synthesis of innate cytokines: pro-inflammatory ($IL_{1\beta}$, IL_6 , IL_8), antiviral (IFN_α , TNF_α) and immuno-regulatory (IL_{12} , IL_{10}) cytokines [5].

In the model, macrophages can be susceptible M_S , phagocytosing M_P , or infected and excreting the virus M_I . We represented the evolution over time of the macrophage concentrations for these three states.

Compared to [1], we grouped the latent and excreting macrophage states into a single infected state.

Decay. All macrophage states are submitted to natural death or/and migration (rate μ_M^{nat}), as well as apoptosis induced by TNF_α (rate μ_M^{ap}) [6]. The natural death rate is considered higher for infected macrophages than for susceptible and phagocytosing macrophages (multiplicative factor δ_μ). Moreover, infected macrophages can be cytolysed by natural killers (rate μ_M^{inn}) and cells from the cellular response (rate μ_M^{ad}).

Recruitment. Susceptible macrophages are recruited from the bloodstream (rate A_m). Cytokines IL_6 and IL_{12} co-amplify the macrophage recruitment in synergy [7, 8, 9]. IL_8 attracts the macrophages to the infection place [9].

Compared to [1], we grouped the pro-inflammatory cytokines $IL_{1\beta}$, IL_6 and IL_8 and hence simplified their action on the macrophage recruitment.

In the absence of virus, the cytokine concentrations are supposed to be negligible and the resulting concentration of susceptible macrophages in the lung is constant: $M_S = M_S^0 = \frac{A_m}{\mu_M^{\text{nat}}}$.

Phagocytosis and infection. When susceptible macrophages encounter free viral particles (V), they can either phagocytose the virus (rate η) or become infected (rate β). We assume that phagocytosing macrophages revert to the susceptible state after viral destruction (rate γ), whereas infected macrophages remain infected (*i.e.* they cannot eliminate the virus). We also assume that the infected and phagocytosing states are exclusive and that once phagocytosing or infected, macrophages cannot phagocytose or be infected by other viral particles [10].

The phagocytosis is amplified by the antiviral cytokines (A_i and IFN_γ) and inhibited by the immuno-modulatory cytokines (IL_{10} , TGF_β). The phagocytosis ending is amplified by the antiviral cytokines and inhibited by IL_{10} . The macrophage infection is amplified by IL_{10} and inhibited by innate antiviral cytokines (A_i) and TGF_β .

Susceptible macrophages:

$$\begin{aligned}
 \dot{M}_S = & A_m [1 + \kappa^+(P_i IL_{12})] && \leftarrow \text{recruitment} \\
 & - \eta M_S V \kappa^-(IL_{10} + TGF_\beta) [1 + \kappa^+(A_i + IFN_\gamma)] && \leftarrow \text{phagocytosis} \\
 & + \gamma M_P \kappa^-(IL_{10}) [1 + \kappa^+(A_i + IFN_\gamma)] && \leftarrow \text{phagocytosis ending} \\
 & - \beta M_S V \kappa^-(A_i + TGF_\beta) [1 + \kappa^+(IL_{10})] && \leftarrow \text{infection} \\
 & - M_S (\mu_M^{\text{nat}} + \mu_M^{\text{inf}} \kappa^+(A_i)) && \leftarrow \text{decay}
 \end{aligned}$$

Phagocytosing macrophages:

$$\begin{aligned}
 \dot{M}_P &= \eta M_S V \kappa^- (\text{IL}_{10} + \text{TGF}\beta) [1 + \kappa^+ (A_i + \text{IFN}_\gamma)] && \leftarrow \text{phagocytosis} \\
 &- \gamma M_P \kappa^- (\text{IL}_{10}) [1 + \kappa^+ (A_i + \text{IFN}_\gamma)] && \leftarrow \text{phagocytosis ending} \\
 &- M_P (\mu_M^{\text{nat}} + \mu_M^{\text{inf}} \kappa^+ (A_i)) && \leftarrow \text{decay}
 \end{aligned}$$

Infected macrophages:

$$\begin{aligned}
 \dot{M}_I &= \beta M_S V \kappa^- (A_i + \text{TGF}\beta) [1 + \kappa^+ (\text{IL}_{10})] && \leftarrow \text{infection} \\
 &- M_I (\mu_M^{\text{nat}} \delta_\mu + \mu_M^{\text{inf}} \kappa^+ (A_i) + \mu_M^{\text{inn}} \text{NK} + \mu_M^{\text{adap}} R_c) && \leftarrow \text{decay \& cytotoxicity}
 \end{aligned}$$

1.4. Viral dynamics

The virus enters the body through the mucosal surfaces of the respiratory tract and replicates in the pulmonary macrophages.

We represented the evolution over time of the free viral particles (V). The inflow of these free viral particles is given by the time-dependent exposure function ($E(t)$). When they encounter susceptible macrophages (M_S), they can either be phagocytosed by the macrophages (rate $u\eta$), resulting in viral destruction, or they can infect the macrophages (rate $u\beta$), resulting in virus replication. Free viral particles are then released in the lung by infected macrophages. The model represents the resultant of the replication within the cell and the release of free viral particles outside the cell, called excretion (rate e). The viral replication is inhibited by antiviral cytokines. Free viral particles can be neutralised by antibodies represented by the humoral response R_h (rate μ_V^{ad}). They are also submitted to natural death and migration outside the lung (rate μ_V^{nat}).

Compared to [1], we considered that the phagocytosis or macrophage infection consume $u\text{TCID}_{50}$ virus per macrophage. As we set $u = 1[\text{TCID}_{50}/\text{cell}]$, this modification finally induced no change. Moreover, the cytokine regulations that applied to the macrophage change of state between the infected latent and excreting states in [1], apply to the viral excretion in this model.

$$\begin{aligned}
 \dot{V} &= E(t) && \leftarrow \text{exposure} \\
 &- u\eta M_S V \kappa^- (\text{IL}_{10} + \text{TGF}\beta) [1 + \kappa^+ (A_i + \text{IFN}_\gamma)] && \leftarrow \text{phagocytosis} \\
 &- u\beta M_S V \kappa^- (A_i + \text{TGF}\beta) [1 + \kappa^+ (\text{IL}_{10})] && \leftarrow \text{infection} \\
 &+ e M_I \kappa^- (A_i + \text{IFN}_\gamma) && \leftarrow \text{excretion} \\
 &- V (\mu_V^{\text{nat}} + \mu_V^{\text{adap}} R_h) && \leftarrow \text{decay \& neutralisation}
 \end{aligned}$$

1.5. Natural killer dynamics

Natural killers are effectors of the innate response. Their main immune functions are the destruction of infected cells and IFN_γ synthesis [11, 5, 9, 12]. These cytotoxic cells are recruited on the infection site by pro-inflammatory cytokines. Their proliferation and immune functions are activated by several cytokines (IFN_γ , IL_{12} , IL_{15} , IL_{18} , IL_{21} , $\text{IFN}_{\alpha\beta}$), whereas IL_{10} inhibits the natural killer differentiation and their immune functions [11].

We represented the dynamics of activated natural killers (NK) and only included the regulations by the most influential cytokines. Their recruitment from the bloodstream (rate α_N) requires pro-inflammatory cytokines P_i and IL_{12} . Natural killers are then activated by IFN_γ and IL_{12} , whereas IL_{10} inhibits the activation. They are submitted to natural death or/and migration (rate μ_R).

Compared to [1], the effects of the pro-inflammatory cytokines on the natural killer recruitment are simplified, as for the susceptible macrophages.

$$\begin{aligned} \dot{NK} &= \alpha_N \kappa^- (\text{IL}_{10}) \kappa^+ (\text{IL}_{12} P_i) \kappa^+ (\text{IL}_{12} + \text{IFN}_\gamma) && \leftarrow \text{recruitment \& activation} \\ &- \mu_R NK && \leftarrow \text{decay} \end{aligned}$$

1.6. Adaptive effector dynamics

The adaptive response activation begins with the naive CD_4^+ T cell differentiation by antigen presenting cells (dendritic cells or macrophages) within the lymphoid tissues. Depending on the cytokine profiles in the lymph nodes, CD_4^+ T cells differentiate into one of three main CD_4^+ effector types, which have specific immune functions: type 1 T helpers (T_{h1} , belonging to the cellular response), type 2 T helpers (T_{h2} , belonging to the humoral response) and regulatory T lymphocytes (T_{reg} , belonging to the regulatory response) [13, 14, 15, 16, 17, 18, 19, 5, 20]. The differentiation towards T_{h1} is induced by IL_{12} and amplified by IFN_γ . The differentiation towards T_{h2} is induced by IL_6 and amplified by IL_4 . The differentiation towards T_{reg} is induced by TGF_β and amplified by IL_{10} . The cytokines responsible for the differentiation towards a CD_4^+ effector type simultaneously inhibit the differentiation towards the two other types. After the differentiation step, the CD_4^+ effectors express their specific immune functions and proliferate. T_{h1} synthesise the IL_2 and IFN_γ cytokines. In addition, they are responsible for the differentiation of the CD_8^+ T cells in cytotoxic lymphocytes (CTL). CTL synthesise IFN_γ and TNF_α and destroy the infected macrophages. T_{h2} synthesise IL_4 and IL_{10} . In addition, they are the main activator of the B lymphocytes (through IL_4 and IL_{10}), which produce the neutralising antibodies. T_{reg} synthesise the TGF_β and IL_{10} immuno-modulatory cytokines. In summary:

- The cellular response: (i) includes T_{h1} and CTL; (ii) is activated by IL_{12} and IFN_γ , inhibited by IL_6 , IL_{10} and TGF_β ; and (iii) is responsible for the synthesis of IL_2 , IFN_γ and TNF_α .
- The humoral response: (i) includes T_{h2} , B lymphocytes and antibodies; (ii) is activated by IL_6 , IL_4 and IL_{10} , inhibited by IL_{12} , IFN_γ and TGF_β ; and (iii) is responsible for the synthesis of IL_4 and IL_{10} and for the viral particle neutralisation.
- The regulatory response: (i) only includes T_{reg} ; (ii) is activated by TGF_β and IL_{10} , inhibited by IL_6 , IL_{12} and IFN_γ ; and (iii) is responsible for the immune response inhibition through its synthesis of the TGF_β and IL_{10} immuno-modulatory cytokines.

In our model, we represented the adaptive response by three effectors corresponding to the three main orientations: cellular (R_c), humoral (R_h) and regulatory (R_r) responses. As for the NK cells, we only represented the dynamics of the activated effectors. Based on the model proposed by Yates et al. (2000) for the regulation of T helper cell populations [20], we synthesised the dynamics of each adaptive effector by three steps: activation by activated macrophages (rate α_R), proliferation (rate p_R) and decay. We represented the regulations of the activation and proliferation steps by the most influential cytokines: IFN_γ , IL_{12} , IL_{10} and TGF_β . The decay includes the natural decay (rate μ_R) and the Activation Induced Cell Death (AICD) induced by the interaction with a T_{h1} from the R_c compartment (rate δ_{R_c}) [20].

Cellular response. [17, 18, 19, 21, 22, 23, 24] R_c represents the type 1 T helper cells and the cytotoxic lymphocytes. Activation is amplified by IFN_γ and IL_{12} and inhibited by IL_{10} . Proliferation is activated by IFN_γ and IL_{12} and inhibited by IL_{10} and TGF_β . R_c synthesises IFN_γ and is responsible for the destruction of infected cells.

$$\begin{aligned} \dot{R}_c &= \alpha_R (Mp + M_I) [1 + \kappa^+ (\text{IFN}_\gamma + \text{IL}_{12})] \kappa^- (\text{IL}_{10}) && \leftarrow \text{activation} \\ &+ p_R R_c \kappa^+ (\text{IFN}_\gamma + \text{IL}_{12}) \kappa^- (\text{IL}_{10} + \text{TGF}_\beta) && \leftarrow \text{proliferation} \\ &- \mu_R R_c - \delta_{R_c} R_c^2 && \leftarrow \text{decay} \end{aligned}$$

Humoral response. [25, 17, 18, 19] R_h represents the type 2 T helper cells, the B lymphocytes and the antibodies. Activation is amplified by IL_{10} and inhibited by IFN_γ and IL_{12} . Proliferation is activated by IL_{10} and inhibited by IFN_γ , IL_{12} and TGF_β . R_h synthesises IL_{10} and is responsible for the neutralisation of free viral particles through antibodies.

$$\begin{aligned}
\dot{R}_h &= \alpha_R (M_P + M_I) [1 + \kappa^+(\text{IL}_{10})] \kappa^-(\text{IFN}_\gamma + \text{IL}_{12}) && \leftarrow \text{activation} \\
&+ p_R R_h \kappa^+(\text{IL}_{10}) \kappa^-(\text{IFN}_\gamma + \text{IL}_{12} + \text{TGF}_\beta) && \leftarrow \text{proliferation} \\
&- \mu_R R_h - \delta_{R_c} R_c R_h && \leftarrow \text{decay}
\end{aligned}$$

Regulatory response. [26, 17, 18, 19] R_r represents the regulatory T cells. Activation is amplified by IL_{10} and TGF_β and inhibited by IFN_γ and IL_{12} . Proliferation is activated by TGF_β and inhibited by IL_{10} , IFN_γ and IL_{12} . R_r synthesises IL_{10} and TGF_β .

$$\begin{aligned}
\dot{R}_r &= \alpha_R (M_P + M_I) [1 + \kappa^+(\text{IL}_{10} + \text{TGF}_\beta)] \kappa^-(\text{IFN}_\gamma + \text{IL}_{12}) && \leftarrow \text{activation} \\
&+ p_R R_r \kappa^+(\text{TGF}_\beta) \kappa^-(\text{IFN}_\gamma + \text{IL}_{12} + \text{IL}_{10}) && \leftarrow \text{proliferation} \\
&- \mu_R R_r - \delta_{R_c} R_c R_r && \leftarrow \text{decay}
\end{aligned}$$

1.7. Cytokine dynamics

Cytokines are small proteins that play a key role in cell-signalling. They are produced by activated immune cells and affect the behaviour of other cells, sometimes the releasing cell itself. They act through specific membranous receptors inducing cascaded reactions within the target cell. They have various functions. In particular, cytokines modulate the balance between the humoral, cellular and regulatory responses. Some cytokines enhance or inhibit the action of other cytokines in complex ways.

In the model, we only integrated the regulations by the most influential cytokines. We grouped them into three classes depending on their main function: pro-inflammatory ($P_i = \text{IL}_{1\beta} + \text{IL}_6 + \text{IL}_8$), antiviral (innate $A_i = \text{TNF}_\alpha + \text{IFN}_\alpha$ and adaptive IFN_γ) and immuno-regulatory (IL_{12} , IFN_γ , IL_{10} and TGF_β) cytokines. TNF_α is generally considered as a pro-inflammatory cytokine, but we were here more interested in its antiviral function. IFN_γ is an antiviral and immuno-regulatory cytokine.

Compared to [1], we represented (i) the pro-inflammatory cytokines $\text{IL}_{1\beta}$, IL_6 and IL_8 by a single variable P_i ; (ii) the innate antiviral cytokines TNF_α and IFN_α by a single variable A_i .

We assumed that the cytokines are efficient enough in order to neglect their consumption when they interact with a cell. So the cytokine dynamics results from their synthesis by immune cells (rates ρ_x^y , where x depends on the cytokine considered and y depends on the producing cells) and their natural death (rate μ_C).

The main cytokine regulations are summarised in Table A1 and the cytokine syntheses in Table A2.

Pro-inflammatory cytokines. [9, 36, 8, 5, 32, 7] $P_i = \text{IL}_{1\beta} + \text{IL}_6 + \text{IL}_8$ amplify the recruitment of macrophages and natural killers. They are synthesised by activated macrophages. The synthesis of $\text{IL}_{1\beta}$ is inhibited by IL_{10} , whereas the synthesis of IL_6 and IL_8 is co-activated by $\text{IL}_{1\beta}$ and TNF_α .

$$\begin{aligned}
\dot{P}_i &= \rho_{P_i} (M_P + M_I) [\kappa^-(\text{IL}_{10}) + 2 \kappa^+(P_i A_i)] && \leftarrow \text{synthesis} \\
&- \mu_C P_i && \leftarrow \text{decay}
\end{aligned}$$

Antiviral cytokines. Antiviral cytokines promote the phagocytosis and reduce the infection by inhibiting the macrophage permissiveness and/or the viral replication (and so the excretion flow in the model).

Innate antiviral cytokines are represented by $A_i = \text{TNF}_\alpha + \text{IFN}_\alpha$. TNF_α is synthesised by activated macrophages, IFN_α by infected macrophages [27, 21, 28, 22, 6, 29, 30, 32, 7]. IL_{10} inhibits the synthesis of TNF_α .

$$\begin{aligned}
\dot{A}_i &= \rho_{A_i} (M_P + M_I) \kappa^-(\text{IL}_{10}) + \rho_{A_i} M_I && \leftarrow \text{synthesis} \\
&- \mu_C A_i && \leftarrow \text{decay}
\end{aligned}$$

Table A1. **Cytokine regulations.** Main up (+) and down (−) regulations included in the model for various immune mechanisms.

	Cytokines					
	pro-inf. P_i^*	innate antiviral A_i^{**}	IFN $_{\gamma}$	IL $_{12}$	IL $_{10}$	TGF $_{\beta}$
<i>Innate response</i>						
Macrophage recruitment	+			+		
NK recruitment & activation	+		+	+	−	
Macrophage apoptosis		+				
Phagocytosis		+	+		−	−
Macrophage permissiveness		−	−		+	−
Viral replication		−	−			
<i>Adaptive response</i>						
Cellular response			+	+	−	−
Humoral response			−	−	+	−
Regulatory response			−	−	±	+
<i>Cytokine syntheses</i>						
Pro-inflammatory P_i	+	+			−	
Innate antiviral A_i					−	
Adaptive antiviral IFN $_{\gamma}$		+	+	+	−	−
Immuno-regulatory IL $_{12}$					−	
IL $_{10}$					+	±
Specific references	[8, 9, 24]	[27, 21, 28, 22, 6, 29, 30]	[21, 22, 23, 24]	[25]	[31, 32, 33, 34, 8, 35, 36]	[26]
Common references		[32, 7]			[17, 18, 19]	
Global references			[5, 37, 38, 39, 15, 16, 14, 13]			

* Pro-inflammatory cytokines $P_i = \text{IL}_{1\beta} + \text{IL}_6 + \text{IL}_8$.** Innate antiviral cytokines $A_i = \text{TNF}_{\alpha} + \text{IFN}_{\alpha}$.Table A2. **Cytokine syntheses.** Production of cytokines included (✓) in the model by innate or adaptive immune cells.

	Cytokines					
	pro-inf. P_i^*	innate antiviral A_i^{**}	IFN $_{\gamma}$	IL $_{12}$	IL $_{10}$	TGF $_{\beta}$
<i>Innate cells</i>						
Activated macrophages	✓	✓		✓	✓	[9, 40] [†]
Activated natural killers			✓			[9, 12] [†]
<i>Adaptive cells</i>						
Cellular effectors			✓			†
Humoral effectors					✓	†
Regulatory effectors					✓	†

* Pro-inflammatory cytokines $P_i = \text{IL}_{1\beta} + \text{IL}_6 + \text{IL}_8$.** Innate antiviral cytokines $A_i = \text{TNF}_{\alpha} + \text{IFN}_{\alpha}$.

† Global references: [5, 41, 42, 15, 16, 18, 37, 14, 13].

The adaptive antiviral cytokine IFN_γ is synthesised by cells of the cellular response (R_c) and natural killers (NK). This synthesis is auto-amplified. It is also amplified by A_i , in synergy with IL_{12} , and inhibited by IL_{10} and TGF_β . IFN_γ is also an immuno-regulatory cytokine and it orientates the adaptive response towards the cellular response [21, 22, 23, 24, 17, 18, 19].

$$\begin{aligned} \dot{\text{IFN}}_\gamma &= (\rho_{\text{IFN}_\gamma}^{\text{inn}} \text{NK} + \rho_{\text{IFN}_\gamma}^{\text{ad}} R_c) [1 + \kappa^+(\text{IL}_{12} A_i) + \kappa^+(\text{IFN}_\gamma)] \kappa^-(\text{IL}_{10} + \text{TGF}_\beta) && \leftarrow \text{synthesis} \\ &- \mu_C \text{IFN}_\gamma && \leftarrow \text{decay} \end{aligned}$$

Immuno-regulatory cytokines. [17, 18, 19, 15, 16, 14, 13] IL_{12} , IFN_γ (see above), IL_{10} and TGF_β have various functions, in particular the regulation of the adaptive immune response. They also regulate the recruitment of macrophages and natural killers, the phagocytosis and infection, as well as the cytokine syntheses. They are produced by cells of the innate and adaptive response.

IL_{12} co-amplifies the recruitment of macrophages, activates the natural killers and orientates the adaptive response towards the cellular response. It is synthesised by activated macrophages. Its synthesis is inhibited by IL_{10} .

$$\begin{aligned} \dot{\text{IL}}_{12} &= \rho_{\text{IL}_{12}} (M_P + M_I) \kappa^-(\text{IL}_{10}) && \leftarrow \text{synthesis} \\ &- \mu_C \text{IL}_{12} && \leftarrow \text{decay} \end{aligned}$$

IL_{10} and TGF_β are both immuno-modulatory cytokines. IL_{10} inhibits the activation of natural killers and the phagocytosis, it amplifies the macrophage permissiveness and it orientates the adaptive response towards the humoral and regulatory responses. IL_{10} is synthesised by activated macrophages and cells of the regulatory (R_r) and humoral (R_h) responses. Its synthesis by macrophages and R_r is amplified by TGF_β , whereas its synthesis by R_h is auto-amplified and inhibited by TGF_β .

$$\begin{aligned} \dot{\text{IL}}_{10} &= (\rho_{\text{IL}_{10}}^{\text{inn}} (M_P + M_I) + \rho_{\text{IL}_{10}}^{\text{ad}} R_r) [1 + \kappa^+(\text{TGF}_\beta)] \\ &+ \rho_{\text{IL}_{10}}^{\text{ad}} R_h [1 + \kappa^+(\text{IL}_{10})] \kappa^-(\text{TGF}_\beta) && \leftarrow \text{synthesis} \\ &- \mu_C \text{IL}_{10} && \leftarrow \text{decay} \end{aligned}$$

TGF_β inhibits the phagocytosis and macrophage permissiveness and orientates the adaptive response towards the regulatory response. In the model, we neglected the synthesis of TGF_β by activated macrophages, so it is only synthesised by cells of the regulatory response.

$$\begin{aligned} \dot{\text{TGF}}_\beta &= \rho_{\text{TGF}_\beta} R_r && \leftarrow \text{synthesis} \\ &- \mu_C \text{TGF}_\beta && \leftarrow \text{decay} \end{aligned}$$

2. Model calibration

The model calibration consisted in the definition of reference scenario, representing the infection of a PRRSv-naive pig at the post-weaning stage with an intermediate susceptibility by an moderately virulent PRRSv strain. The parameter values hence defined are listed in Table A4 and are based on the reference scenario used in the previous model [1]. We present here the calibration method used in our previous work.

Available data. As we did not have access to specific experimental data to calibrate our model, we performed an extensive literature review and collected published data from both experimental and modelling studies.

- **On the parameter values.** Most immunological mechanisms are difficult to monitor because of technical constraints and there are consequently not enough experimental data to directly inform all the parameter values. So we collected parameter values used in published models applied to pathogens targeting antigen presenting cells, including the PRRS virus, *Mycobacterium Tuberculosis* and the influenza virus. We hence defined rather large ranges for our parameter values.
- **On the model variables.** Then we compiled information on the dynamics of the model variables (viral titer and immune component concentrations) from published studies on PRRSv experimental infections, reviewed in [37, 43, 44, 45, 46, 47, 48, 49, 22]. Experimental protocols exhibit a strong variability and may differ on: the monitoring duration, the immune components measured, the titration method for a given component, the viral strain, the pig genotype and the pig age. This variability results in heterogeneous outputs among experimental studies, which is illustrated for the viral titer, the best informed variable in the literature, in Table A3. Moreover, among the variables included in our model, only a few were monitored in each experimental study and there were few measures over time. So we synthesised these data to set criteria for realistic model outputs (viral titer and immune component dynamics) [50, chapter 1].

Calibration method. Based on the available data described above, classical parameter estimation methods were not suitable to calibrate our model and we had to design an *ad hoc* procedure to estimate the model parameters. This procedure consisted of three steps:

- (i) Exploration of the parameter space defined by the parameter ranges obtained from modelling studies on pathogens targeting antigen presenting cells. Illustrations are available in [1, supplementary figures].
- (ii) Selection of a reference parameter set resulting in realistic model outputs, based on the criteria defined from published experimental data. If we were able to define quantitative criteria for the viral titer, we mainly extracted qualitative criteria for the immune component dynamics because of the strong heterogeneity and sparsity of published experimental data. Among the parameter sets meeting these criteria, we selected a reference parameter set corresponding to an average response of an experimentally infected and isolated pig to a moderately virulent PRRSv strain. More details on the choice of the reference scenario are provided in [1].
- (iii) Global sensitivity analyses on the model outputs. Such analyses were performed to identify the most influential parameters on the model outputs, which consequently need to be precisely informed. They were also used to check the model robustness to parameter uncertainty, *i.e.* that reasonable variations of parameter values still result in realistic model outputs. The global sensitivity analysis method and the results are presented in [1].

Model outputs for the reference scenario. Reference scenario outputs for the previously published model [1] and the simplified version presented here are represented in Figure A2. They are very close. The viral titers, our first criterion for the model calibration, are almost identical in both models (Figure A2.A). The other well-preserved model outputs are the phagocytosing macrophages (Figure A2.C), the humoral and regulatory effectors (Figure A2.G & H) and the cytokine TGF_{β} (Figure A2.N). Reference scenarios mostly differ in the natural killers (Figure A2.E), the adaptive cellular effectors (Figure A2.F) and the cytokine IFN_{γ} (Figure A2.L), *i.e.* in the global cellular response level (lower for the simplified model). Whatever the model output, the differences are quantitative (peak value) rather

Table A3. **Synthesis of PRRSV viral titer characteristics from published experimental data**, which monitored during at least 14 days post infection (dpi). The variation ranges among published studies of three quantitative characteristics of the viral titer were extracted: the infection duration D_I (in dpi), the viral peak V_{\max} (in $\log(\text{TCID}_{50}/\text{ml})$) and the date of the viral peak T_{\max} (in dpi). These characteristics are sorted depending on the PRRSV strains (type 1 European or type 2 American genotype) used in the studies. We also specified the main outlines of the experimental protocols which can be involved in the observed variability between studies: the **pig breed** and **ages** at infection (in days), the **sample origin** (blood or broncho-alveolar lavage), the viral **titration method** and the **inoculation dose** (in $\log(\text{TCID}_{50}/\text{ml})$) and **route**.

Strain x breed x age			Viral titer characteristics			Sample origin	Titration method	Inoculation		Ref.
Strain	Breed	Age	D _I	V _{max}	T _{max}			dose	route	
Type 1 European genotypes										
Lelystad	Ω	27 – 49	≥ 21 – 52	2 – 7.3	6 – 14	Ω	Ω	5 – 6	Ω	[51, 52, 53, 54, 55]
Belgium A	Ω	42	21 – ≥ 35	2.4 – 4.6	10	blood	Ω	5 – 6	IN	[53, 56]
SDRP-15D	∅	56	21 – 49	2.8 – 3.7	14	blood	VI	4	IN-IV	[57]
3262	∅	28	14 – 35	2.5 – 3.7	7	blood	VI	5.7	IN	[58]
3267	∅	28	42 – 63	3.4 – 4	7	blood	VI	5.7	IN	[58]
2982	∅	35 – 45	14 – ≥ 24	2.3 – 4.5	14	blood	VI	3	IM	[59, 60]
Lena	Ω	42	21 – ≥ 35	3.8 – 6.1	10 – 14	blood	Ω	5 – 6	IN	[53, 56]
Type 2 American genotypes										
VR-2332	Ω	14-42	> 21 – 112	1 – 6.3	7 – 14	Ω	Ω	3.4 – 5	Ω	[23, 61, 62, 63]
Ingelvac MLV	Ω	14 – 35	9 – 72	0.8 – 3.7	6 – 12	blood	Ω	3.4 – 5	Ω	[61, 64]
JA-142	Ω	14 – > 140	11 – 36	2.8 – 4.5	1 – 5	blood	Ω	3 – 4.3	Ω	[61, 65, 66]
Ingelvac ATP	Ω	14 – > 140	14 – 63	0.8 – 3.5	3 – 21	blood	Ω	3 – 4.5	Ω	[61, 66]
97-7985	Ω	18 – 29	> 14 – > 42	3.7 – 7	4 – 14	blood	Ω	2 – 5.7	Ω	[54, 67, 68]
SDSU-73	Ω	14 – 35	> 35	3.5 – 4.3	5 – 7	blood	VI	2.8 – 4.3	IN	[61, 65]
Abst-1	Co	14 – 21	7	0.2	3	blood	VI	4.2	IN	[61]
MN-184	Co	14 – 21	35	4.6	7	blood	VI	4.1	IN	[61]
17198-6	Co	14 – 21	35	2.8	15	blood	VI	2.8	IN	[61]
16244 B	∅	27	> 21	3.2 – 3.8	6 – 9	blood	VI	5.7	IN	[54]
761040 0	∅	27	> 21	3.7 – 4.7	9	blood	VI	5.7	IN	[54]

∅: not informed in the published studies.
Ω: variable protocol outlines between studies of PRRSv experimental infection by a given viral strain. See **Table I.2** in [50] for details.
Pig breed: various (Ω) among Landrace, Large White, Yorkshire, Pietrin, Miniature, Hampshire, Duroc, NE Index line, conventional (Co) or cross-breed
Viral titration method: various (Ω) among viral isolation (VI), quantitative RT-PCR or Immuno Fluorescence Monolayer Assay
Inoculation route: various (Ω) among intranasal (IN), intramuscular (IM), intranasal and intravenous (IN-IV), intranasal and intramuscular or through aerosol

Table A4. **Model parameters for the reference scenario.**

Parameter	Description	Value	Unit*
Macrophages: susceptible (M_S), infected (M_I), phagocytosing (M_P)			
A_m	recruitment rate of M_S	$5 \cdot 10^4$	$[\text{Ce}] \text{day}^{-1}$
η	phagocytosis rate	$5 \cdot 10^{-7}$	$[\text{V}]^{-1} \text{day}^{-1}$
β	infection rate	10^{-6}	$[\text{V}]^{-1} \text{day}^{-1}$
γ	1/phagocytosis duration	96	day^{-1}
μ_M^{nat}	natural decay rate	0.2	day^{-1}
δ_μ	over-mortality rate of M_I	1.1	no unit
μ_M^{ap}	apoptosis rate by TNF_α	0.8^\dagger	day^{-1}
μ_M^{inn}	cytolysis rate of M_I by NK	10^{-3}	$[\text{Ce}]^{-1} \text{day}^{-1}$
μ_M^{ad}	cytolysis rate of M_I by R_c	10^{-3}	$[\text{Ce}]^{-1} \text{day}^{-1}$
Free viral particles (V)			
u	macrophage-virus interaction rate	1	$[\text{V}][\text{Ce}]^{-1}$
e	excretion rate	0.2	$[\text{V}][\text{Ce}]^{-1} \text{day}^{-1}$
μ_V^{nat}	natural decay rate	0.2	day^{-1}
μ_V^{ad}	neutralisation rate by R_h	10^{-4}	$[\text{Ce}]^{-1} \text{day}^{-1}$
Adaptive effectors: cellular (R_c), humoral (R_h), regulatory (R_r) & natural killers (NK)			
α_R	activation rate of R_c , R_h and R_r by M_P and M_I	10^{-5}	day^{-1}
α_N	activation rate of NK	10	$[\text{Ce}] \text{day}^{-1}$
p_R	proliferation rate of R_c , R_h and R_r	0.05	day^{-1}
δ_{R_c}	decay rate of R_c , R_h and R_r by AICD	10^{-1}	$[\text{Ce}]^{-1} \text{day}^{-1}$
μ_R	natural decay rate	0.05	day^{-1}
Cytokines: pro-inflammatory ($P_i = \text{IL}_{1\beta} + \text{IL}_6 + \text{IL}_8$), innate antiviral ($A_i = \text{TNF}_\alpha + \text{IFN}_\alpha$), immuno-regulatory ($\text{IL}_{12}, \text{IFN}_\gamma, \text{IL}_{10}, \text{TGF}_\beta$)			
ρ_{P_i}	synthesis rate of P_i by M_P and M_I	10^{-2}	$[\text{Cy}][\text{Ce}]^{-1} \text{day}^{-1}$
$\rho_{\text{IL}_{12}}$	synthesis rate of IL_{12} by M_P and M_I	10^{-2}	$[\text{Cy}][\text{Ce}]^{-1} \text{day}^{-1}$
ρ_{A_i}	synthesis rate of A_i by M_P and M_I	0.05	$[\text{Cy}][\text{Ce}]^{-1} \text{day}^{-1}$
$\rho_{\text{IL}_{10}}^{\text{inn}}$	synthesis rate of IL_{10} by M_P and M_I	0.02	$[\text{Cy}][\text{Ce}]^{-1} \text{day}^{-1}$
$\rho_{\text{IL}_{10}}^{\text{ad}}$	synthesis rate of IL_{10} by R_h and R_r	2	$[\text{Cy}][\text{Ce}]^{-1} \text{day}^{-1}$
$\rho_{\text{IFN}_\gamma}^{\text{inn}}$	synthesis rate of IFN_γ by NK	10	$[\text{Cy}][\text{Ce}]^{-1} \text{day}^{-1}$
$\rho_{\text{IFN}_\gamma}^{\text{ad}}$	synthesis rate of IFN_γ by R_c	10	$[\text{Cy}][\text{Ce}]^{-1} \text{day}^{-1}$
ρ_{TGF_β}	synthesis rate of TGF_β by R_r	10	$[\text{Cy}][\text{Ce}]^{-1} \text{day}^{-1}$
μ_C	natural decay rate	20	day^{-1}
k_m	half-saturation concentration	30	$[\text{Cy}]$
v_m	saturation factor	1.5	no unit
*cell unit $[\text{Ce}] = \text{cells/ml}$, virus unit $[\text{V}] = \text{TCID}_{50}/\text{ml}$, cytokine unit $[\text{Cy}] = \text{pg/ml}$			
† updated parameter value			

than qualitative (output shape). Furthermore, our reference scenario does not correspond to a particular data set, but to an average within-host dynamics obtained from the synthesis of experimental data. Both reference scenarios are consistent with literature data (*cf.* **Table 5** in the main text). Consequently, the qualitative behaviour of the model is well-preserved and we believe that the reference scenario defined for the simplified version of the model is satisfying.

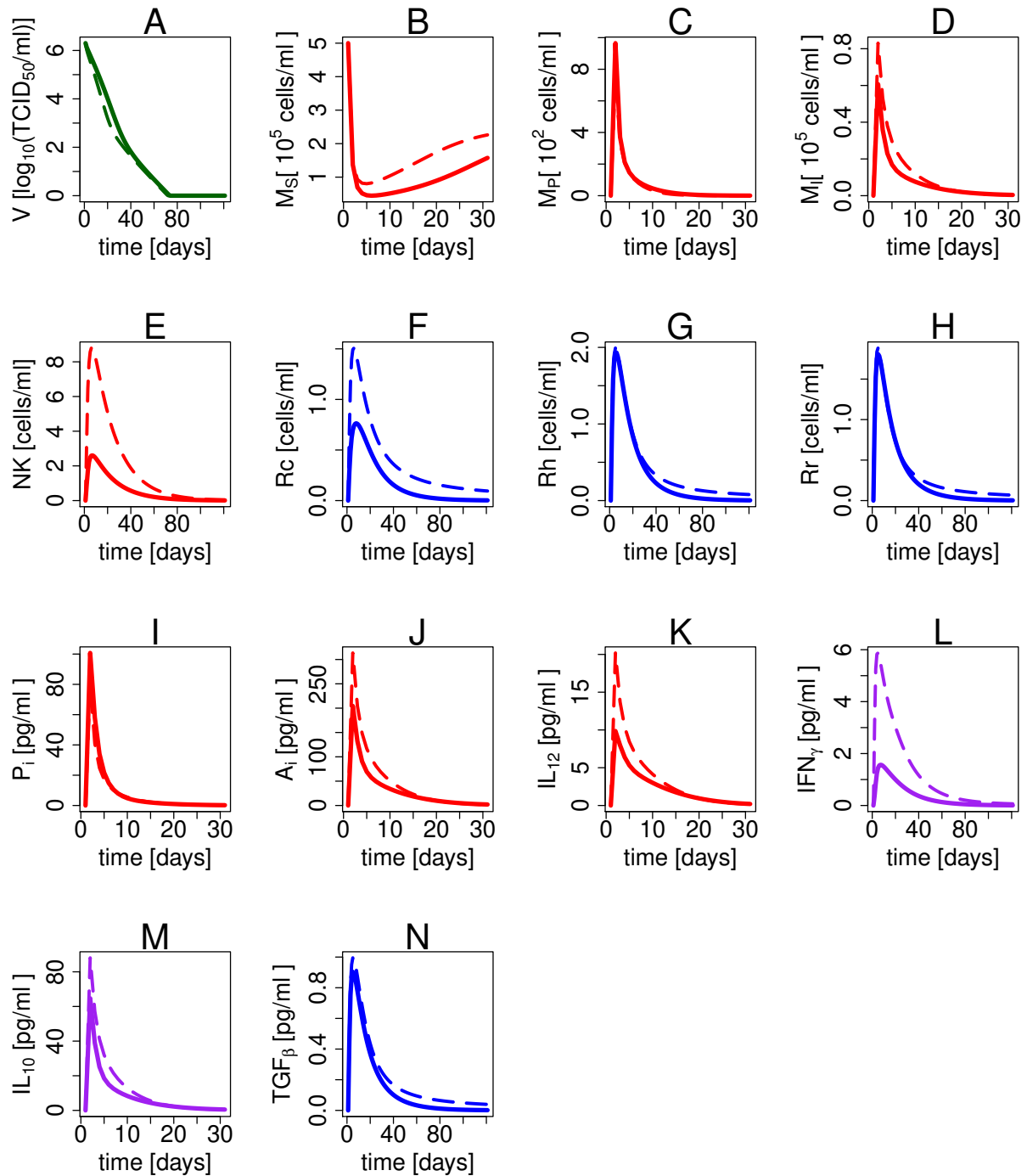


Figure A2. **Model outputs for the reference scenario: comparison between the Lung-based model (dashed lines) [1] and the simplified version of this model presented here (solid lines).**

Model outputs:

- viral titer (V in green, figure A);
- innate effectors (in red): susceptible (M_S , subfigure B), phagocytosing (M_P , subfigure C) or infected (M_I , subfigure D) macrophages and natural killers (NK, subfigure E);
- adaptive effectors (in blue) of the cellular (R_C , subfigure F), humoral (R_h , subfigure G) or regulatory (R_r , subfigure H) responses;
- cytokines synthesised by innate effectors (in red): pro-inflammatory (P_i , subfigure I), innate antiviral (A_i , subfigure J), immuno-regulatory IL_{12} (subfigure K);
- cytokines synthesised by adaptive effectors (in blue): immuno-modulatory TGF_β (subfigure N);
- cytokines synthesised by both innate and adaptive effectors (in purple): antiviral IFN_γ (subfigure L) and immuno-modulatory IL_{10} (subfigure M).

3. Combined influence of within-host mechanisms and exposure on the viral load

By model construction, (see **Model description**), the viral load results from the viral exposure (income of viral particles inside the host) and the within-host dynamics (infection and immune mechanisms). Our results show that both exposure and within-host mechanisms have a notable influence on the viral load (see **Results** section in the main text). Figure A3 below complements these results: it highlights the influence of immune mechanisms on the viral dynamics and it illustrates the non-linear relations between the viral load of the recipient and the exposure.

Method. We defined a set of three sub-models associated with a gradual level of complexity in the representation of the within-host mechanisms:

1. neither infection nor immune mechanisms, *i.e.* the sub-model only represents the viral exposure and the viral natural decay (red curves on Figure A3);
2. infection but no immune mechanisms, *i.e.* the sub-model represents the viral exposure, the viral natural decay, the target cell recruitment (baseline rate, no cytokine regulation), the target cell infection and the viral replication (blue curves on Figure A3);
3. infection and immune mechanisms, *i.e.* the sub-model corresponds to the model presented in the paper (green curves on Figure A3).

As the strength of some immune mechanisms varies depending on the virulence level (S_{vir}), we ran the three sub-models above for each of the three contrasted levels of virulence that we explored in our paper: low (S_{vir1} , first column on Figure A3), medium (S_{vir2} , second column on Figure A3) and high (S_{vir3} , third column on Figure A3). In order to highlight the influence of the exposure dose on the viral dynamics, we combined the three scenario of virulence level with three contrasted exposure doses among the one tested in the paper: low (L_1 , first row on Figure A3), medium (L_3 , second row on Figure A3) and high (L_6 , third row on Figure A3). We did not vary the exposure duration, fixed at an intermediate value of 20 days.

Results. Figure A3 shows that, whatever the scenario [virulence level x exposure dose], the infection duration notably differs between the three sub-models. This finding illustrates that the infection resolution simulated by the model presented in the paper (sub-model 3 here) is not only determined by the exposure dose, but rather results from the combination of the exposure dose, infection mechanisms and immune mechanisms. Infection durations either resulting from the model presented in the paper (sub-model 3, green curves) or the sub-model 2 (blue curves), which accounts for the infection mechanisms but not the immune mechanisms, are particularly contrasted. This highlights the major role played by the immune mechanisms on the infection duration.

Moreover, Figure A3 shows that the earlier dynamics of the viral load is similar for the three sub-models and high exposure doses (L_6 , last row), but not for low exposure doses. This corresponds to a global saturation effect of the immune and infection mechanisms for high exposure doses, as highlighted in the paper results.

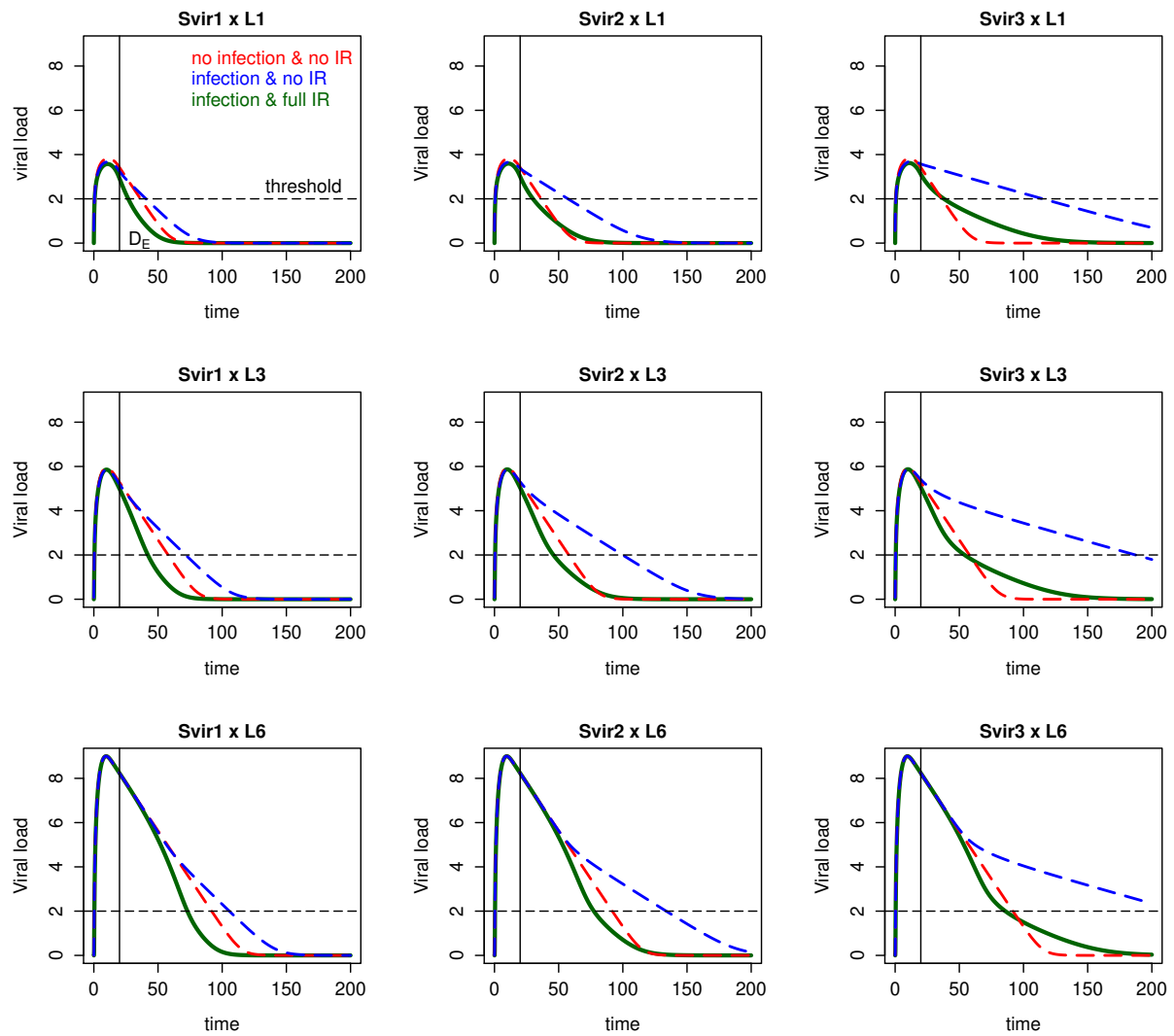


Figure A3. **Combined influence of the within-host mechanisms and the exposure dose on the viral load.** Viral load over infection time (semi-log graph) generated from three sub-models (colours) for 9 contrasted scenarios of virulence level \times exposure dose [virulence level: low: Svir1, medium: Svir2, high: Svir3 – columns ; exposure dose: low: L1, medium: L3, high: L6 – lines].

SUB-MODELS – 1. **Neither infection nor immune mechanisms:** viremia = exposure - natural decay. 2. **Infection but no immune mechanisms:** viremia = exposure - natural decay + replication; target cells = recruitment - natural decay; infected cells = cell infection - natural decay. 3. **Infection and immune mechanisms:** model presented in the paper.

Vertical line: exposure duration ($D_E=20$ days).

Horizontal dashed line: viral detection threshold (10^2 TCID₅₀/ml).

References

- [1] N. Go, C. Bidot, C. Belloc, S. Touzeau, Integrative model of the immune response to a pulmonary macrophage infection: what determines the infection duration?, *PLoS ONE* 9 (9) (2014) e107818. doi:10.1371/journal.pone.0107818.
- [2] D. Gammack, S. Ganguli, S. Marino, J. Segovia-Juarez, D. Kirschner, Understanding the immune response in tuberculosis using different mathematical models and biological scales, *Multiscale Model. Sim.* 3 (2) (2005) 312–345. doi:10.1137/040603127.
- [3] S. Marino, A. Myers, J. L. Flynn, D. E. Kirschner, TNF and IL-10 are major factors in modulation of the phagocytic cell environment in lung and lymph node in tuberculosis: A next-generation two-compartmental model, *J. Theor. Biol.* 265 (4) (2010) 586–598. doi:10.1016/j.jtbi.2010.05.012.
- [4] J. E. Wigginton, D. Kirschner, A model to predict cell-mediated immune regulatory mechanisms during human infection with *Mycobacterium tuberculosis*, *J. Immunol.* 166 (2001) 1951–1967.
- [5] B. T. Rouse, S. Sehrawat, Immunity and immunopathology to viruses: what decides the outcome?, *Nat. Rev. Immunol.* 10 (7) (2010) 514–526. doi:10.1038/nri2802.
- [6] C. Choi, C. Chae, Expression of tumour necrosis factor alpha is associated with apoptosis in lungs of pigs experimentally infected with porcine reproductive and respiratory syndrome virus, *Res. Vet. Sci.* 72 (1) (2002) 45–49. doi:10.1053/rvsc.2001.0519.
- [7] K. Van Reeth, S. Van Gucht, M. Pensaert, In vivo studies on cytokine involvement during acute viral respiratory disease of swine: troublesome but rewarding, *Vet. Immunol. Immunop.* 87 (3–4) (2002) 161–168. doi:10.1016/S0165-2427(02)00047-8.
- [8] A. J. Roth, E. L. Thacker, Immune system, in: B. E. Straw, J. J. Zimmerman, S. D’Allaire, D. L. Taylor (Eds.), *Diseases of swine*, ninth Edition, Blackwell, Oxford, UK, 2006, Ch. 2, pp. 15–36.
- [9] M. F. Tosi, Innate immune responses to infection, *J. Allergy Clin. Immun.* 116 (2) (2005) 241–249. doi:10.1016/j.jaci.2005.05.036.
- [10] R. Thanawongnuwech, Pulmonary intravascular macrophages (PIMs): What do we know about their role in PRRSV infection in pigs, *Wetchan Sattawaphaet (The Thai journal of veterinary medicine)* 29 (1999).
- [11] A. L. DeFranco, R. M. Locksley, M. Robertson, R. Cunin, *Immunité : la réponse immunitaire dans les maladies infectieuses et inflammatoires*, 2nd Edition, DeBoeck, Bruxelles, Belgium, 2009.
- [12] S. M. Vidal, S. I. Khakoo, C. A. Biron, Natural killer cell responses during viral infections: flexibility and conditioning of innate immunity by experience, *Curr. Opin. Virol.* 1 (6) (2011) 497–512. doi:10.1016/j.coviro.2011.10.017.
- [13] A. A. T. M. Bosch, G. Biesbroek, K. Trzcinski, E. A. M. Sanders, D. Bogaert, Viral and bacterial interactions in the upper respiratory tract, *PLoS Pathog.* 9 (1) (2013) e1003057. doi:10.1371/journal.ppat.1003057.
- [14] P. Borghetti, Cell-mediated immunity and viral infection in pig, in: PRRS fatti vs speculazioni, Università degli Studi di Parma, Dipartimento di Salute Animale, Parma, Italy, 2005, pp. 27–46.
URL http://medvet.unipr.it/cgi-bin/campusnet/documenti.pl/Show?_id=7d0d
- [15] T. J. Braciale, J. Sun, T. S. Kim, Regulating the adaptive immune response to respiratory virus infection, *Nat. Rev. Immunol.* 12 (4) (2012) 295–305. doi:10.1038/nri3166.
- [16] C. Coquerelle, M. Moser, DC subsets in positive and negative regulation of immunity, *Immunol. Rev.* 234 (1) (2010) 317–334. doi:10.1111/j.0105-2896.2009.00887.x.
- [17] P. Kidd, Th1/Th2 balance: the hypothesis, its limitations, and implications for health and disease, *Altern. Med. Rev.* 8 (3) (2003) 223–246.
- [18] T. LeRoith, S. A. Ahmed, Regulatory T cells and viral disease, in: M. Khatami (Ed.), *Inflammation, Chronic Diseases and Cancer: Cell and Molecular Biology, Immunology and Clinical Bases*, InTech, Rijeka, Croatia, 2012, Ch. 6, pp. 121–144. doi:10.5772/28502.
- [19] C. A. Knosp, J. A. Johnston, Regulation of CD4+ T-cell polarization by suppressor of cytokine signalling proteins, *Immunology* 135 (2) (2012) 101–111. doi:10.1111/j.1365-2567.2011.03520.x.
- [20] A. Yates, C. Bergmann, J. L. Van Hemmen, J. Stark, R. Callard, Cytokine-modulated regulation of helper T cell populations, *J. Theor. Biol.* 206 (4) (2000) 539–560. doi:10.1006/jtbi.2000.2147.
- [21] L. Lopez-Fuertes, E. Campos, N. Domenech, A. Ezquerro, J. M. Castro, J. Dominguez, F. Alonso, Porcine reproductive and respiratory syndrome (PRRS) virus down-modulates TNF- α production in infected macrophages, *Virus Res.* 69 (269) (1999) 41–46. doi:10.1016/S0168-1702(00)00172-6.
- [22] J. Zimmerman, D. A. Benfield, M. P. Murtaugh, F. Osorio, G. W. Stevenson, M. Torremorell, Porcine reproductive and respiratory syndrome virus (porcine arterivirus), in: B. E. Straw, J. J. Zimmerman, S. D’Allaire, D. L. Taylor (Eds.), *Diseases of swine*, ninth Edition, Blackwell, Oxford, UK, 2006, Ch. 24, pp. 387–418.
- [23] R. M. Molina, S.-H. Cha, W. Chittick, S. Lawson, M. P. Murtaugh, E. A. Nelson, J. Christopher-Hennings, K.-J. Yoon, R. Evans, R. R. Rowland, J. J. Zimmerman, Immune response against porcine reproductive and respiratory syndrome virus during acute and chronic infection, *Vet. Immunol. Immunop.* 126 (2008) 283–292. doi:10.1016/j.vetimm.2008.08.002.
- [24] J. K. Lunney, E. R. Fritz, J. M. Reecy, D. Kuhar, E. Prucnal, R. Molina, J. Christopher-Hennings, J. Zimmerman, R. R. Rowland, Interleukin-8, interleukin-1 β , and interferon- γ levels are linked to PRRS virus clearance, *Viral Immunol.* 23 (2) (2010) 127–134. doi:10.1089/vim.2009.0087.
- [25] R. Thanawongnuwech, A. Rungsipipat, S. Disatian, R. Saiyasombat, S. Napakanaporn, P. G. Halbur, Immunohistochemical staining of IFN γ positive cells in porcine reproductive and respiratory syndrome virus-infected lungs, *Vet. Immunol. Immunop.* 91 (1) (2003) 73–77. doi:10.1016/S0165-2427(02)00268-4.
- [26] J. Gómez-Laguna, F. J. Salguero, F. J. Pallarés, L. Carrasco, Immunopathogenesis of porcine reproductive and respiratory syndrome in the respiratory tract of pigs, *Vet. J.* 195 (2) (2013) 148–155. doi:10.1016/j.tvjl.2012.11.012.
- [27] E. Albina, Le point sur le dernier-né des arterivirus : le virus du syndrome dysgénésique et respiratoire porcin (SDRP), *Virologie* 4 (2) (2000) 113–121.
- [28] M. P. Murtaugh, PRRSV/host interaction, in: PRRS fatti vs speculazioni, Università degli Studi di Parma, Dipartimento di Salute Animale, Parma, Italy, 2005, pp. 73–80.
URL http://medvet.unipr.it/cgi-bin/campusnet/documenti.pl/Show?_id=7d0d

- [29] L. C. Miller, W. W. Laegreid, J. L. Bono, C. G. Chitko-McKown, J. M. Fox, Interferon type I response in porcine reproductive and respiratory syndrome virus-infected MARC-145 cells, *Arch. Virol.* 149 (2004) 2453–2463. doi:10.1007/s00705-004-0377-9.
- [30] M. Gimeno, L. Darwich, I. Díaz, E. de la Torre, J. Pujols, M. Martin, S. Inumaru, E. Cano, M. Domínguez, M. Montoya, E. Mateu, Cytokine profiles and phenotype regulation of antigen presenting cells by genotype-I porcine reproductive and respiratory syndrome virus isolates, *Vet. Res.* 42 (9) (2011). doi:10.1186/1297-9716-42-9.
- [31] H. C. Chang, Y. T. Peng, H. L. Chang, H. C. Chaung, W. B. Chung, Phenotypic and functional modulation of bone marrow-derived dendritic cells by porcine reproductive and respiratory syndrome virus, *Vet. Microbiol.* 129 (3–4) (2008) 281–293. doi:10.1016/j.vetmic.2007.12.002.
- [32] Y.-T. Peng, H.-C. Chaung, H.-L. Chang, H.-C. Chang, W.-B. Chung, Modulations of phenotype and cytokine expression of porcine bone marrow-derived dendritic cells by porcine reproductive and respiratory syndrome virus, *Vet. Microbiol.* 136 (3–4) (2009) 359–365. doi:10.1016/j.vetmic.2008.11.013.
- [33] D. Yoo, C. Song, Y. Sun, Y. Du, O. Kim, H.-C. Liu, Modulation of host cell responses and evasion strategies for porcine reproductive and respiratory syndrome virus, *Virus Res.* 154 (1–2) (2010) 48–60. doi:10.1016/j.virusres.2010.07.019.
- [34] L. Flores-Mendoza, E. Silva-Campa, M. Reséndiz, F. A. Osorio, J. Hernández, Porcine reproductive and respiratory syndrome virus infects mature porcine dendritic cells and up-regulates interleukin-10 production, *Clin. Vaccine Immunol.* 15 (2008) 720–725. doi:10.1128/CVI.00224-07.
- [35] S. Suradhat, R. Thanawongnuwech, Y. Poovorawan, Upregulation of IL-10 gene expression in porcine peripheral blood mononuclear cells by porcine reproductive and respiratory syndrome, *J. Gen. Virol.* 84 (2003) 453–459. doi:10.1099/vir.0.18698-0.
- [36] K. W. Moore, R. de Waal Malefyt, R. L. Coffman, A. O’Garra, Interleukin-10 and the interleukin-10 receptor, *Annu. Rev. Immunol.* 19 (1) (2001) 683–765. doi:10.1146/annurev.immunol.19.1.683.
- [37] L. Darwich, I. Díaz, E. Mateu, Certainties, doubts and hypotheses in porcine reproductive and respiratory syndrome virus immunobiology, *Virus Res.* 154 (1–2) (2010) 123–132. doi:10.1016/j.virusres.2010.07.017.
- [38] M. P. Murtaugh, Z. Xiao, F. Zuckermann, Immunological responses of swine to porcine reproductive and respiratory syndrome virus infection, *Viral Immunol.* 15 (4) (2002) 533–547. doi:10.1089/088282402320914485.
- [39] J. Gómez-Laguna, F. J. Salguero, I. Barranco, F. J. Pallares, I. M. Rodríguez-Gómez, A. Bernabe, L. Carrasco, Cytokine expression by macrophages in the lung of pigs infected with the porcine reproductive and respiratory syndrome virus, *J. Comp. Pathol.* 142 (1) (2009) 51–60. doi:10.1016/j.jcpa.2009.07.004.
- [40] D. M. Underhill, H. S. Goodridge, Information processing during phagocytosis, *Nat. Rev. Immunol.* 12 (2012) 492–502. doi:10.1038/nri3244.
- [41] S. Akira, K. Takeda, T. Kaisho, Toll-like receptors: critical proteins linking innate and acquired immunity, *Nat. Immunol.* 2 (8) (2001) 675–680. doi:10.1038/90609.
- [42] G. C. Sen, Viruses and interferons, *Annu. Rev. Microbiol.* 55 (2001) 255–281. doi:10.1146/annurev.micro.55.1.255.
- [43] L. Darwich, M. Gimeno, M. Sibila, I. Díaz, E. de la Torre, S. Dotti, L. Kuzemtseva, M. Martin, J. Pujols, E. Mateu, Genetic and immunobiological diversities of porcine reproductive and respiratory syndrome genotype I strains, *Vet. Microbiol.* 150 (1–2) (2011) 49–62. doi:10.1016/j.vetmic.2011.01.008.
- [44] T. G. Kimman, L. A. Cornelissen, R. J. Moormann, J. M. J. Rebel, N. Stockhofe-Zurwieden, Challenges for porcine reproductive and respiratory syndrome virus (PRRSV) vaccinology, *Vaccine* 27 (28) (2009) 3704–3718. doi:10.1016/j.vaccine.2009.04.022.
- [45] J. K. Lunney, H. Chen, Genetic control of host resistance to porcine reproductive and respiratory syndrome virus (PRRSV) infection, *Virus Res.* 154 (1–2) (2010) 161–169. doi:10.1016/j.virusres.2010.08.004.
- [46] E. Mateu, I. Díaz, The challenge of PRRS immunology, *Vet. J.* 177 (2008) 345–351. doi:10.1016/j.tvjl.2007.05.022.
- [47] M. P. Murtaugh, M. Genzow, Immunological solutions for treatment and prevention of porcine reproductive and respiratory syndrome (PRRS), *Vaccine* 29 (46) (2011) 8192–8204. doi:10.1016/j.vaccine.2011.09.013.
- [48] H. J. Nauwynck, H. Van Gorp, M. Vanhee, U. Karniychuk, M. Geldhof, A. Cao, M. Verbeeck, W. Van Breedam, Micro-dissecting the pathogenesis and immune response of PRRSV infection paves the way for more efficient PRRSV vaccines, *Transbound. Emerg. Dis.* 59 (2012) 50–54. doi:10.1111/j.1865-1682.2011.01292.x.
- [49] R. Thanawongnuwech, S. Suradhat, Taming PRRSV: Revisiting the control strategies and vaccine design, *Virus Res.* 154 (1–2) (2010) 133–140. doi:10.1016/j.virusres.2010.09.003.
- [50] N. Go, *Modelling the immune response to the Porcine Respiratory and Reproductive Syndrome virus*, Ph.D. thesis, L’institut des Sciences et Industries du Vivant et de l’Environnement (AgroParisTech) (2014). URL <https://hal.inria.fr/tel-01100983/document>
- [51] G. G. Labarque, H. J. Nauwynck, K. Van Reeth, M. B. Pensaert, Effect of cellular changes and onset of humoral immunity on the replication of porcine reproductive and respiratory syndrome virus in the lungs of pigs, *J. Gen. Virol.* 81 (5) (2000) 1327–1334.
- [52] X. Duan, H. J. Nauwynck, M. B. Pensaert, Virus quantification and identification of cellular targets in the lungs and lymphoid tissues of pigs at different time intervals after inoculation with porcine reproductive and respiratory syndrome virus (PRRSV), *Vet. Microbiol.* 56 (1–2) (1997) 9–19. doi:10.1016/S0378-1135(96)01347-8.
- [53] E. Weesendorp, S. Morgan, N. Stockhofe-Zurwieden, D. J. Graaf, S. P. Graham, J. M. J. Rebel, Comparative analysis of immune responses following experimental infection of pigs with European porcine reproductive and respiratory syndrome virus strains of differing virulence, *Vet. Microbiol.* 163 (2013) 1–12. doi:10.1016/j.vetmic.2012.09.013.
- [54] F. J. Martínez-Lobo, F. Díez-Fuertes, J. Segalés, C. García-Artiga, I. Simarro, J. M. Castro, C. Prieto, Comparative pathogenicity of type 1 and type 2 isolates of porcine reproductive and respiratory syndrome virus (PRRSv) in a young pig infection model, *Vet. Microbiol.* 154 (1) (2011) 58–68. doi:10.1016/j.vetmic.2011.06.025.
- [55] S. B. Morgan, S. P. Graham, F. J. Salguero, P. J. Sánchez Cordón, H. Mokhtar, J. M. J. Rebel, E. Weesendorp, K. B. Bodman-Smith, F. Steinbach, J. P. Frossard, Increased pathogenicity of European porcine reproductive and respiratory syndrome virus is associated with enhanced adaptive responses and viral clearance, *Vet. Microbiol.* 163 (1) (2013) 13–22. doi:10.1016/j.vetmic.2012.09.013.
- [56] U. Karniychuk, M. Geldhof, M. Vanhee, J. Van Doorsselaere, T. Saveleva, H. Nauwynck, Pathogenesis and antigenic characterization of

- a new East European subtype 3 porcine reproductive and respiratory syndrome virus isolate, *Vet. Res.* 6 (1) (2010) 30. doi:10.1186/1746-6148-6-30.
- [57] E. Albina, L. Pirou, E. Hutet, R. Cariolet, R. L'Hospitalier, Immune response in pigs infected with porcine reproductive and respiratory syndrome virus (PRRSV), *Vet. Immunol. Immunop.* 61 (1998) 49–66. doi:10.1016/S0165-2427(97)00134-7.
- [58] I. Díaz, M. Gimeno, L. Darwich, N. Navarro, L. Kuzemtseva, S. López, I. Galindo, J. Segalés, M. Martín, J. Pujols, E. Mateu, Characterization of homologous and heterologous adaptive immune responses in porcine reproductive and respiratory syndrome virus infection, *Vet. Res.* 43 (1) (2012) 30. doi:10.1186/1297-9716-43-30.
- [59] J. Gómez-Laguna, F. Salguero, F. Pallarés, M. Fernández de Marco, I. Barranco, J. Cerón, S. Martínez-Subiela, K. Van Reeth, L. Carrasco, Acute phase response in porcine reproductive and respiratory syndrome virus infection, *Comp. Immunol. Microb.* 33 (6) (2010) e51–e58. doi:10.1016/j.cimid.2009.11.003.
- [60] J. Gómez-Laguna, I. M. Rodríguez-Gomez, I. Barranco, F. J. Pallarés, F. J. Salguero, L. Carrasco, Enhanced expression of TGF β protein in lymphoid organs and lung, but not in serum, of pigs infected with a European field isolate of porcine reproductive and respiratory syndrome virus, *Vet. Microbiol.* 158 (1) (2012) 187–193. doi:10.1016/j.vetmic.2012.02.003.
- [61] W. Johnson, M. Roof, E. Vaughn, J. Christopher-Hennings, C. R. Johnson, M. P. Murtaugh, Pathogenic and humoral immune responses to porcine reproductive and respiratory syndrome virus (PRRSV) are related to viral load in acute infection, *Vet. Immunol. Immunop.* 102 (3) (2004) 233–247. doi:10.1016/j.vetimm.2004.09.01.
- [62] A. B. Doeschl-Wilson, I. Kyriazakis, A. Vincent, M. F. Rothschild, E. Thacker, L. Galina-Pantoja, Clinical and pathological responses of pigs from two genetically diverse commercial lines to porcine reproductive and respiratory syndrome virus infection, *J. Anim. Sci.* 87 (2009) 1638–1647. doi:10.2527/jas.2008-1447.
- [63] P. Mulupuri, J. J. Zimmerman, J. Hermann, C. R. Johnson, J. P. Cano, W. Yu, S. A. Dee, M. P. Murtaugh, Antigen-specific B-cell responses to porcine reproductive and respiratory syndrome virus infection, *J. Virol.* 82 (1) (2008) 358–370. doi:10.1128/JVI.01023-07.
- [64] G. Reiner, C. Fresen, S. Bronnert, H. Willems, Porcine reproductive and respiratory syndrome virus (PRRSV) infection in wild boars, *Vet. Microbiol.* 136 (3) (2009) 250–258. doi:10.1016/j.vetmic.2008.11.023.
- [65] D. R. Wesley, K. M. Lager, M. E. Kehrli, Infection with porcine reproductive and respiratory syndrome virus stimulates an early gamma interferon response in the serum of pig, *Can. J. Vet. Res.* 70 (3) (2006) 176–182.
- [66] K. L. Klinge, E. M. Vaughn, M. B. Roof, E. M. Bautista, M. P. Murtaugh, Age-dependent resistance to porcine reproductive and respiratory syndrome virus replication in swine, *Virol. J.* 6 (1) (2009) 177. doi:10.1186/1743-422X-6-177.
- [67] D. B. Petry, J. Lunney, P. Boyd, D. Kuhar, E. Blankenshi, R. K. Johnson, Differential immunity in pigs with high and low responses to porcine reproductive and respiratory syndrome virus infection, *J. Anim. Sci.* 85 (2007) 2075–2092. doi:10.2527/jas.2006-721.
- [68] N. Boddicker, E. H. Waide, R. R. Rowland, J. K. Lunney, D. J. Garrick, J. M. Reecy, J. C. M. Dekkers, Evidence for a major QTL associated with host response to porcine reproductive and respiratory syndrome virus challenge, *J. Anim. Sci.* 90 (6) (2012) 1733–1746. doi:10.2527/jas.2011-4464.