Dear Dr. Magalhaes

We appreciate the opportunity to resubmit a revised version of our manuscript entitled "**Coevolution of virulence and immunosuppression through multiple infections".**

We have upload the revised version on bioRxiv:
https://www.biorxiv.org/content/early/2017/10/24/149211

Please find below detailed responses to reviewer comments.

Thank you,

Tsukushi Kamiya, Nicole Mideo and Samuel Alizon

Revision *by Sara Magalhaes, 2017-07-14 12:26*
Decision & reviews
The article presents a model analyzing the co-evolution of immunosuppression and virulence under multiple infections. This represents a very important contribution for the existing models on both the evolution of virulence under multiple infections and the evolution of virulence in immunosuppressing parasites (with the latter being clearly less explored than the former). The model is very well-designed and provides valuable insight into this important question. However, the reviewers and myself found that the presentation of several results was hard to follow. Also (I do not discard that this may be related to the previous sentence) some parameter choices seem rather arbitrary, or at least unjustified. I understand that the issue at stake is a complex one, with several factors operating simultaneously. However, it is important for the reader to take some message home… This is compromised at several instances, figure 3 being a paradigmatic example of this (cf. comments by rev1). On a more positive note, I think the discussion does a good job in summarizing the main findings. Overall, I'm convinced of the high quality of this manuscript, but would urge the authors to consider the reviewers comments to improve the clarity of their message. Below I also present my own comments.

Main comment: As mentioned in lines 187-190 and 211-216, and recapitulated in the Discussion (lines 282-284) double infections are protected from further infections. In my opinion, this may be the crucial factor of your model. That is, would you allow for triple infections (or more than that), wouldn't high ESI at low virulence pattern vanish? Actually, the situation found here is much akin to models of niche construction (immunosuppression can be considered as a case of niche construction), in which the evolution of this trait is favored if niche constructers also evolve means to monopolize the resource (e.g., Krakaeur et al. 2009 Am Nat). Maybe establishing this parallel would be useful?

> **The parallel is interesting but the problem is that here what we have is more of a physical constraint rather than a modification by a particular parasite strain. As discussed in earlier articles (e.g. Alizon et al. 2013), the problem comes from the fact that the coinfection model we use (which derives from van Baalen & Sabelis 1995) makes the assumption that the two parasite strains studies are very close, this allowing us to simplify the more complicated model (studied by Choisy & de Roode 2010). In this latter model, the limitation would appear more clearly: the**

host can only be infected by one strain of each parasite species and the first one to infect cannot be ousted from the host. When simplifying the Choisy and de Roode model, we get the host class of host coinfected by the same resident strain that emerges.

In addition to the presentation of the framework itself, we have rethought our protection interpretation and think that there is a more parsimonious explanation as to the dynamics observed when varying mortality rate.

We now make the comparison between the two models clear by including a schematic of how van Baalen & Sabelis model is a special case of the Choisy and de Roode model.

Minor comments: Line 15: The existence of different host types comes as a surprise… which types of hosts are there?

In response to the comment of the second reviewer that the sentence in Line 15 was repetitive, we have removed the sentence.

Line 27: any reason not to cite Van Baalen and Sabelis 1995 here?

We have added the suggested citation.

Lines 31-34: I find the transition quite abrupt here. In the abstract you mention that most models do not consider the coevolution of traits in parasites. Maybe you could explore this a bit more here before turning to the particular traits you will tackle?

What we meant was the coevolution between traits controlled by the parasite. There are indeed many coevolution models where one of the traits is under the control of the parasite and another is under the control of the host.

Lines 35-43: I know this is a bit biased, but if you would cite some examples from the plant-parasite literature, you would also attract more attention from that community, which unfortunately often neglects host-parasite theory. Eg Sarmento et al. Ecol Lett 2011, Burgyan and Havelda 2011 Trends Plant Sci. This can also be done in the Discussion section.

The citations are now added along with a new sentence "Also in plant parasites, a variety of mechanisms exist to suppress host defensive responses [burgyan2011viral, sarmento2011herbivore]" (L. 40)

Lines 79-80: equations: why is the recovery rate of doubly infected to single infected the double of that from singly infected to susceptible (2 γ Drr vs γ Ir)?

In our model, parasites are assumed to be cleared independently. Therefore, a host infected with two parasites is twice as likely to clear one parasite as a host infected with one parasite.

Line 159: “convergently stable”, instead of “convergent stable” (cf also relevant comments of rev1 on this part).

Any mention of “convergent stable” is now changed to “convergently stable”.

Line 166: I would remove “game theoretically”.

We have removed the phrase.

Line 177: remove “assumption”.

Removed.

Figure 1: Is there a specific reason for not exploiting the whole range of virulence? Especially, the slight increase observed for higher virulence values makes us wonder what will happen for even higher values. Figs 1d,e: I understand space is limited but maybe stating ‘relative frequency of (co)infected hosts’ in the Y axis would be much more intuitive…

The maximum virulence we explore (i.e., 0.5 per day meaning that parasite induced mortality on average kills an infected host in 2 days) is already very high biologically. Increasing it further increasing it further would only remove the cost of immunosuppression (no risk to be coinfected) and the trend of increasing immunosuppression would continue.

Also please consider a comment by rev1 on the trade-off values adopted here.

Please see below.

Note also that the δ values stated here differ from those in table 1.

Thank you for pointing out the typo. We have changed the values in the table.

Lines 187-190: this section needs some streamlining, as the information on low virulence being correlated with more double infections is provided twice.

We have rephrased the sentences (L. 183-191).

Reviewed by anonymous reviewer*, 2017-07-04 13:27*
I found the paper interesting and well-written, and I enjoyed reading it.
One concern I have though is that Figure 3 and the results it presents are quite hard to parse for the readers.
The color levels are not explained in the legend or in the Figure: what is being plotted?

The figure caption is now changed to describe the plot more precisely: "The shade of blue and red indicates the co-evolutionarily singular strategy value of (a) immunosuppression and (b) virulence, respectively".

The default set of parameters (used in all results and figures before) appears quite atypical: quite decelerating for one trade-off and quite decelerating for the other. What motivates this particular choice? Why not take, if any, a "simple" reference point such as linear/linear? It is very important that the readers understand to what extent all that is said in the first part of the results (Figs 1& 2) is robust to the trade-off curves, and not specific to the values 0.25 and 0.05 that were chosen.

The choice of the trade-off parameters is indeed a valid concern. In fact, we precisely included Figure 3 in the main text to show the reader the importance of this assumption.

The reviewer is right in that our choice for the default parameter values were too implicit. The parasite strategy (immunosuppression) acts on two infection traits, one that improves parasite fitness (decreased host recovery) and another that decreases it (increased sensitivity to infection).

Our assumption is that the recovery trade-off is decelerating and the susceptibility trade-off is accelerating. The reason for this is that it is usually observed when there are trade-offs that the more a beneficial trait is increased, the more it becomes costly to increase it further. For the costly trait, the choice matters less (as shown in Figure 3). We chose an accelerating cost to further emphasize the difference between the beneficial and costly traits.

We now further explain our default concavity parameter choices based on the explanations above (L. 124-126).

Also for clarity, we modified a Figure S1 to include the default trade-off shapes.

Figure 3 should be made more readable but otherwise makes a good job at showing how the trade-off parameters affect evolutionary dynamics. However, it only reports evolutionary stability (ESS / convergence stability), not the location of the (co)ESS.
I would expect the location of the co-ESS, which is obviously the main focus of the article, to receive the same treatment and be explored over a range of tradeoff shapes.

As it stands, the first part details results on coESS location for a specific set of trade-off functions, and then we get a generalization to other tradeoff functions, but only for evolutionary stability. It seems that the section about trade-off shape and evolutionary stability, restricted in fact to Figure 3 and to less than 20 lines at the end of the Results (l. 220-236) are quite disconnected. And the relative length of the latter part, especially considering the more complex Figure 3, make it underrepresented. This is also appearant in the Discussion, where line 253 we just have one sentence "In addition, immunosuppression evolution is influenced considerably by the precise shape of the trade- offs determining the cost and benefit of immunosuppression" to

sum up these findings. I think the authors should adopt the same approach (i.e. consider a range of trade-off shpes) for all results (both the location of the (co)ESS and the evolutionary stability), and also restore some balance between the attention given to evolutionary stability versus ESS location. Considering there are only three figures, there is ample space for one or two additional figures, if needed (e.g. if a large part of the results are worked out for a specific shape of tradeoff functions, then it can be worth plotting these specific fucntions as the firt figure; currently we only have a general presentation of all possible tradeoff shapes and it is only in the Supp. Material).

> **Here perhaps we were not explicit enough in the colour code of the figure: the color refers to the trait values at the co-ESS if it exists. If it does not exist, we use a special code (black or white squares).**
>
> **To clarify this, we have done the following. First, we have now added "co-ESV" and "co-ESI" next to the legends for the colours in the grid. Second, the figure caption is now changed to describe the plot more precisely: "The shade of blue and red indicates the co-evolutionarily singular strategy value of (a) immunosuppression and (b) virulence, respectively".**

I also have some more technical questions/remarks on the model and its presentation:
-a- On multiple infection and omission of D*mm*: the authors motivate this omission from the rarity of the mutant. This is a common assumption when individuals mix randomly and thus the probability of encountering another mutant is vanishnigly small. However, in this model, what is a multiple infection exactly? Can a secondary infection originates from the host itself? I mean, if multiple infections are infections at different parts of the body or different tissues, could not a virus reinfect its own host? I would think of left lung infection to right lung infection in humans, or infection of a different master twig in a tree-crown. This would actually be the most likely route to multiple infection considering the physical proximity. When this is the case, the fact that a mutant is very rare does not compromise the rate of multiple infection so much, and so *D*mm sould not be neglected. I presume the authors have in mind that a second mutant should necessarily come from a different host, which motivates the assumption, but why would it be necesssarily so?

Perhaps the authors can elaborate on motivating their assumption, in relation to the biological mechanisms considered. I think more generally the definition of a multiple infection deserves some attention/explanation. Indeed, the examples provided are mostly for very different infectious agents (e.g. the helminth that favors microparasites through immunosuppression, l. 48). In contrast, if I understand the model considers very similar (or perfectly similar) strains of the same pathogen: D*rr or D*mm denote double infection from the same variant, end even r and m are marginally different variants. In this context, how could D*mm not be the same as D*m? My understanding is that this implies that we can still discriminate the two m populations in D*mm, which would in turn mean (if they are genetically identical) that they form two physically distinct subpopulations in one same host (and see the previous paragraph on what this would imply for the omission of D*mm). Otherwise, it would mean that adding a very small amount of propagule from the same genotype would considerably alter the within-host population dynamics, which is not very intuitive to me.

I am sure the authors can clarify all these points and discuss them, and the key is to specify more precisely what is the within-host population dynamics. This is important because several modelling assumptions (and some results it seems) hinge on that: for instance, the fact that \alpha*rr is the same as \alpha*r, or that \alpha*rm is the average of the \alpha, suggests that the total load of virus in the host does not quite depend on the number of infections. That \beta is simply divided between the two strains in a host in proportion to their relative virulences also points to the same direction. But can't we imagine that the two infections are somewhat different in their location, and thus that a doubly infected host suffers more overall? To make it clearer: if the within-host dynamics is governed by pure resource competition, as the authors say, then either the two strains will not persist within the host (no coexistence) and two identical infections would not cause a great difference compared to a single infection (nothing would select for the second infection to increase in frequency within the host). The fact that this is not the case (coexistence + quantitative difference between the single and doubly infected hosts) implies on the contrary some form of niche differentiation (spatial, temporal or whatever) of pathogen populations within an individual host. This in turn would lead to the possibilities I mention above (D*mm not negligible, or \alpha*rr > \alpha*r). I would also think that imposing a strong limit on double infections (i.e. no host can be infected more than twice), even when, in the model simulations, most hosts can be in the twice-infected state (Figures 1 and 2), is strange if there is no niche differentiation of the consecutive infections (e.g., there are no more than two lungs in a body, to follow on my earlier example). Otherwise, multiple infections could readily occur and consecutive infections would add up into the total population as is modelled here. A common motivation to "cut" the vector of multiple infections is when multiple infections are rare (and thus beyond two infections, we can neglect the events). But this is obviously not the case in this model.
I do not ask the authors to provide an explicit equation for the dynamics of resource and competition within a host, but simply to clarify the type of within-host interactions at play and how the different assumptions would result from this interaction (especially since some sentences in the Discussion revolve around these issues).

There are many issues raised here so we apologize in advance if we missed some.

On the first part of the question, yes, as in most multiple infection models, we assume that an inoculation event is necessary to move from a single to a multiple infection. With this assumption, the doubly infection by a mutant become negligible due to the assumption of rarity of the mutant.

To be more specific, we think that here part of the problem originates from the counter-intuitive setting of the van Baalen & Sabelis coinfection model. As explained above, this model is a simplification of a coinfection model with 2 species (Choisy & de Roode 2010). In such a setting, the two parasites can be as different as a bacterium and a virus. Furthermore, the model does not allow two different bacterial strain to coinfect the same host. Put differently, there cannot be any local colonisation because there is only one site available in the host for each parasite species. This limitation is still present in our model, where we assume that the two parasite species from Choisy & de Roode are identical (to simplify the calculations). We now highlight the parallel between the two models in Figure 1.

Overall, our model is different in essence from a model where there would be two patches in each host that can be colonised via local or distant dispersal, which is why we do not investigate it. Notice that on such an issue, classical island models could provide good insight because they elegantly deal with these local/distant colonisation events.

About our assumptions regarding the overall virulence (i.e. that it is the average of the two coinfecting strains), it turns out we could make other assumptions (e.g. summing the virulences) without violating the assumptions of the framework (see Alizon 2013, *Interface Focus*). We chose the average because of our assumption that the mutant and the resident are similar, and hence they share the same resource.

About the transmission rate from coinfected hosts, we apologize for not being more explicit in the main text: we assume that this transmission rate is constant in the default case, i.e. there is always the same number of propagules that are emitted per unit of time from an infected host. The assumption that the transmission rate is constant is made clear in Table 1.

Finally, on the more general question about within-host dynamics, we completely agree that in reality most of the between-host assumptions we make, starting with the ability for the two strains to coexist in a host, originate from within-host dynamics. This is why we tried to remain as general as possible in our hypotheses. Clearly, adapting this model to a specific host-parasite interaction with an explicit within-host model would be extremely valuable!

-b- Why is the clearance rate \gamma is assumed to be the same for two strains competing in a host? If the two strains differ in virulence, and thus have different transmission rates from the host (\beta), I expect their respective loads differ within the host, so that the "rarer" variant (rare because of competition from the other variant) might also be more susceptible to disappear from the host?

This is a good point: we here assume that the competition between the strains will affect their relative transmission from the host but that their risk of being cleared is the same. It could be possible to assume that the risk of clearance also depends on the virulence of each strain. From a mathematical standpoint, this would of course complexify the model greatly and we think that to be addressed satisfyingly, it would require an explicit modeling of the within-host dynamics. From a biological standpoint, we here consider the case with a unique resident species and a mutant that is very similar to the resident. Therefore, it makes sense that the immune response faced by all parasite strains is similar.

We now mention that adding a within-host component to the model would allow to improve the mechanistic aspects, especially have the virulence, transmission, immunosuppression and recovery functions depend on parasite load (L. 289-291).

-c- In Section S3, when introducing a virulence-transmission tradeoff, how exactly is the function \beta(x,c) combined with eq (2) in the main text? The base model assumes the two strains share a common pie (\beta) in proportion to x, but how do you do this in the more complex model where the two pies differ in size? Please clarify.

We now provide an explicit description of the transmission rate (Supporting Information equation 4).

-d- On the formatting of equations and presentation of model: in many cases, parameters are represented by one letter without a subscript, which strongly suggests they are constant, and it is only much alter that we learn that, in fact, the parameter is not constant but is dependent of various things. This is confusing and should be changed so that we can immediately see which parameters are the same or may differ. Typically, in eq (1), it looks like \alpha and \gamma will be constant, as it is not subscripted. It is not clear until much later (e.g. eq (5-6)) that the two will vary. The same thing holds for \beta, actually.

We have modified our expression to demonstrate that susceptibility to coinfection and recovery are functions of immunosuppression, and that virulence is a function of parasite exploitation (equation 1).

-e- On the definition of the fitness and adaptive dynamics: the section presenting the fitness R and its subsequent use for evolutionary analysis (page 10) is extremely elusive. I could not find anywhere what R looks like exactly. It is mentioned that it is obtained from a local stability analysis, but I'd like to see more details, and perhaps an expression for R (which is usually feasible in those types of models, as a R0-like metric), that I could not find in the main document or in the Supp. Material.

We have added the mutant invasion fitness expressions (equation 6).

In the same vein, it is stated l. 94 that the equilibrium can be obtained analytically, but this is shown nowhere and never used: either show the result, or the statement can as well be dropped.

The phrase is now dropped.

Also, the authors never model the joint evolution of the two traits but perform two one-trait ESS analyses and "superpose" them. Did they check the convergence stability in two-dimension? Is the co-ESS a stable node or a focus? Can there be evolutionary cycles?

We now check for the convergence stability in two-dimension using the approach of Abrams et al. 1993 and Marrow et al. 1996.

-f- On line 159, the conditions for convergence stability and branching are exchanged: the first condition b<0 ("R is at a local maximum") is for evolutionary stability and the impossibility of

being invaded, whereas the second a-b>0 is for convergence stability, contrary to what is written.

We have rephrased the explanation of evolutionary stability and convergence stability

TYPOS and other side points
-- Line 195: " focusing on the prevalence of co-infections alone is not enough to predict how ESI will evolve." What do you mean exactly by prevalence of co-infections? Do you mean the ratio D/(D+I) or just D? It seems that the ration D/(D+I) is stricttly decreasing with virulence, and that the switch in the gradient of Immuno Suppression coincides with a 50:50 ratio (D/I=1), so that the latter has some utility to predict the change in ESI.

l. 97: the first sentence of this section is not very clear, please reformulate.

We agree with the reviewer that the sentence was confusing. We rephrased the sentence as follows: “It is commonly assumed that virulence (i.e., parasite-induced host mortality) correlates with the extent of parasite resource exploitation. Adaptive benefits of resource exploitation include the positive correlation with transmission [alizon2009virulence}], and within-host competitive advantage in co-infection [van1995dynamics,choisy2010mixed]. Here, we focus on the latter adaptive benefit to study the evolution of virulence and immunosuppression.”

l. 104: the more virulENT strains...
l. 20 in Supp. Material: The Figure called should be S2, not S1
l. 38 in Supp. Material: same thing

We appreciate the reviewer for pointing out these errors. We have now corrected them.

***Reviewed by anonymous reviewer**, 2017-07-07 01:55*
In this work, the authors apply an adaptive dynamics framework based on epidemiological models to find how the presence of immunosuppression affects virulence evolution in the context of multiple infections. As a result they find that by increasing the opportunity of co-infections, the presence of immunosuppression leads to ESS with higher virulence levels. This effect is modulated by factors such as the benefit from reduced clearance or host background mortality such that the optimal immunosuppression will depend on virulence in a non-simple way and also on the specific trade-offs between recovery rate and increased susceptibility of hosts to co-infections. This is a very interesting work and the approach taken, even if based on a specific set of epidemiological assumptions, adequate to produce results of relevance to the study of evolution of pathogenesis.
I only have some comments regarding parts of the manuscript where more details are needed or clarifications should be given.

1.Lines 15-16 partially repeat lines 12-14 in abstract.

We now deleted the repetitive sentence.

2.The placement of equations 2 in the text is awkward. Those formulas are only mentioned much later in the text.
Even if the base model is already described in Alizon 2008, and given the importance of the epidemiological assumption of the Drr individuals, the comparison between their resident dynamics to the mutant system should be made more explicit. For instance, it is stated that: "We treat the host classs Drr similarly to singly infected hosts, Ir, except for the fact that the doubly infected hosts cannot be infected any further." (lines 93-95) I guess this only refers to to the risk of contracting a further infection. What about mortality rate? From line 141 one would conclude that this is resolved by assuming a mean alpha of the infecting strains, which seems to differ from Lipsitch et al 2009 approach.

We now better explain how the model we use is derived from a more general version (see Figure 1). In another study (Alizon 2013 Interface Focus), it is shown that the actual definition of virulence (mean of the virulence of the two strains or sum of the two) does not affect the approach itself. The choice should therefore depend on the biology. Here, since we consider microparasites and assume that mutant and resident are very similar, it made more sens to use the definition with the average.

Related to this, the authors have probably checked that the augmented models (equations 4 and equations 5a and 5b) lead to the same equillibria as the one with just the resident allele (equations 1), if the mutant allele is absent. If so, this should be mentioned.

Here we are not sure what the reviewer has in mind. Equations 4 and 5/6 are for invasion purposes only. In fact, earlier work has shown that since the mutant is assumed to be very similar to the resident, both cannot coexist (van Baalen & Sabelis 1995). Therefore, there would not be a stable state with all host types for a system combining equations 1 and 4.

The equilibrium demographics under immunosuppression are only shown in the context of co-evolutionary stable immunosuppression (co-ESI) or co-evolutionary stable virulence. But how much those demographics differ from the ones obtained under the resident strain only?

Here we are not sure that we understand the concern.

In this model, there cannot be any long term coexistence of a resident and a mutant strain (see van Baalen & Sabelis 1995 Am Nat). This originates from the fact that the two are assumed to be very similar. Therefore, any equilibrium demographics can only be obtained with a single (resident) strain.

That being said, in the demographics we show in Figure 1, when we vary virulence (panels b & d) or immunosuppression (panels c & e), we do assume that the other

trait is sitting as its ESS. Representing the dynamics for all the trait values would be extremely difficult and we do not think it would bring additional insights.

Why aren't the formulas for the dynamics of the susceptible individuals shown for the mutant systems? Given the focus that is given later on the demographics of the different individual types (particularly Fig 2) it would be better if those would be included (even with the cost of some repetition)

We apologize for not being clearer in the writing. When introducing a mutant, i.e. when equations 4a & b are relevant, we assume that the resident system is at equilibrium. Therefore, we assume that equation system 1 is active and that all the equations are equal to 0 (because S, I_r, D_rr are all at equilibrium).

When showing the demographics, we show the equilibrium values (time on the x axis). We modified the caption of Figure 2 to clarify this issue.

6.Regarding virulence evolution (Figure 1), why is that at immunosuppression approaching 100% there is a sharp increase in the ESV? What are the equilibrium frequencies of S,I and D in that situation? Does it still conform to the explanation that the increase in ESV is due to more opportunity for within-host competition as proposed by the authors?

This is a very accurate observation! Indeed, there is a steep increase in ESV when immunosuppression is maximal (and therefore when infected hosts never clear the infection given our definition of the trade-off between immunosuppression and recovery rate).

The fact that the increase in virulence (Fig 2a) correlates with the increase in the proportion of infections that are coinfections (Fig 2e) is consistent with the idea that these are the drivers of virulence. However, we do not see such an acceleration in the proportion of coinfections when immunosuppression reaches 100%.

Unfortunately, we do not have an intuitive explanation for the sharp increase in virulence but we should bear in mind that this is not a coevolutionary state (we're forcing the immunosuppression remain maximal whatever the costs for the parasite), which could generate unexpected feedbacks.

For the immunosuppression evolution. When virulence is increased is just the virulence of the resident strain (and the mean virulence, as expected from page 9) while maintaining the virulence of possible mutants the same?

When only immunosuppression evolves, we assume that virulence remains constant for the whole evolutionary analysis (i.e. the successive replacement of

residents by mutants until we reach an ESS, i.e. a state where the resident cannot be invaded).

So to answer the question more directly, yes the virulence is the same for the resident and the mutants: they only differ in their immunosuppression.

Line 211. Force of infection or virulence?

We have entirely restructured the paragraph in question (L. 205-222).

The way the whole paragraph that includes lines 210 to 219 is presented is somewhat confusing. I guess this stems from not being immediately clear to which part of the graph the authors are referring to in lines 211 to 213 (it must be the left part of the graphs in Figure 2).

We agree that our writing was ambiguous. We have therefore entirely restructured the paragraph in question (L. 205-222).

Color scale in Figure 3 refers to ESI (a) and ESV (b) obtained, but this not clear in the Figure or Figure legend.

The figure caption is now changed to describe the plot more precisely: "The shade of blue and red indicates the co-evolutionarily singular strategy value of (a) immunosuppression and (b) virulence, respectively".