Peer Community In Evolutionary Biology

A new hypothesis to explain Ebola's high virulence

Virginie Ravigné and *François Blanquart* based on peer reviews by *François Blanquart* and *Virginie Ravigné*

Mircea T. Sofonea, Lafi Aldakak, Luis Fernando Boullosa, Samuel Alizon (2017) Can Ebola Virus evolve to be less virulent in humans? Missing preprint_server, ver. Missing article_version, peer-reviewed and recommended by Peer Community in Evolutionary Biology. 10.1101/108589

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The tragic 2014-2016 Ebola outbreak that resulted in more than 28,000 cases and 11,000 deaths in West Africa [1] has been a surprise to the scientific community. Before 2013, the Ebola virus (EBOV) was known to produce recurrent outbreaks in remote villages near tropical rainforests in Central Africa, never exceeding a few hundred cases with very high virulence. Both EBOV's ability to circulate for several months in large urban human populations and its important mutation rate suggest that EBOV's virulence could evolve and to some extent adapt to human hosts [2]. Up to now, the high virulence of EBOV in humans was generally thought to be maladaptive, the virus being adapted to circulating in wild animal populations (e.g. fruit bats [3]). As a logical consequence, EBOV virulence could be expected to decrease during long epidemics in humans. The present paper by Sofonea et al. [4] challenges this view and explores how, given EBOV's life cycle and known epidemiological parameters, virulence is expected to evolve in the human host during long epidemics. The main finding of the paper is that there is no chance that EBOV's virulence decreases in the short and long terms. The main underlying mechanism is that EBOV is also transmitted by dead bodies, which limits the cost of virulence. In itself the idea that selection should select for higher virulence in diseases that are also transmitted after host death will sound intuitive for most evolutionary epidemiologists. The accomplishment of the paper is to make a very strong case that the parameter range where virulence could decrease is very small. The paper further provides scientifically grounded arguments in favor of the safe management of corpses. Safe burial of corpses is culturally difficult to impose. The present paper shows that in addition to instantaneously decreasing the spread of the virus, safe burial may limit virulence increase in the short term and favor of less virulent strains in the long term. Altogether these results make a timely and important contribution to the knowledge and understanding of EBOV.

References:

[1] World Health Organization. 2016. WHO: Ebola situation report - 10 June 2016.

[2] Kupferschmidt K. 2014. Imagining Ebola's next move. Science 346: 151–152. doi: [10.1126/science.346.6206.151](https://doi.org/10.1126/science.346.6206.151)

[3] Leroy EM, Kumulungui B, Pourrut X, Rouquet P, Hassanin A, Yaba P, Délicat A, Paweska, Gonzalez JP and Swanepoel R. 2005. Fruit bats as reservoirs of Ebola virus. Nature 438: 575–576. doi: [10.1038/438575a](https://doi.org/10.1038/438575a)

[4] Sofonea MT, Aldakak L, Boullosa LFVV and Alizon S. 2017. Can Ebola Virus evolve to be less virulent in humans? bioRxiv 108589, ver. 3 of 19th May 2017; doi: [10.1101/108589](https://doi.org/10.1101/108589)

Reviews

Evaluation round #2

DOI or URL of the preprint: **10.1101/108589**
Version of the preprint: 2

Authors' reply, 19 May 2017

The authors have convincingly addressed most comments. A very few number of points (raised by the two referees) need to be corrected on the preprint. Then, it would be a pleasure to recommend it for PCI Evol Biol. Thanks a lot ! **Reviewed by François Blanquart**

Figure 4 I see the colored area on the biorxiv preview of the pdf, but not when opening the downloaded pdf with mac preview

We have used several pdf readers and have never seen this problem. We are sad to admit that we do not know how to solve it.

Below equation 8 define E dot, I dot, S, E dot, H dot, D dot, C dot...

Done.

line 313 "it the infectious dead bodies" -> incomplete

It is now rewritten.

what I meant regarding line 310-312 and the reference to the Bolker and Berngruber papers: mention (if relevant) the specific effect of transient evolution for further virulence in an expanding epidemic (transient effect at out-of-equilibrium demographic dynamic). I was thinking this may explain the large increase in virulence that you see on the first 300 days of figure 5B. (0 to 300 days is the period where the epidemic is expanding, figure 1).

We have rewritten this paragraph following your advice. **Reviewed by Virginie Ravigné**

The authors have convincingly addressed most comments. Please correct the following points: L15 typo is still there L313 word missing (it the infectious bodies) L357 "maximised for maximum CFR" sounds odd I do not need to see the paper again and wish to recommend it.

Done.

Decision by Virginie Ravigné, posted 19 May 2017

Modifications required

The authors have convincingly addressed most comments. A very few number of points (raised by the two referees) need to be corrected on the preprint. Then, it would be a pleasure to recommend it for PCI Evol Biol.

Reviewed by François Blanquart, 19 May 2017

Figure 4 I see the colored area on the biorxiv preview of the pdf, but not when opening the downloaded pdf with mac preview

Below equation 8 define E dot, I dot, S, E dot, H dot, D dot, C dot...

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what I meant regarding line 310-312 and the reference to the Bolker and Berngruber papers: mention (if relevant) the specific effect of transient evolution for further virulence in an expanding epidemic (transient effect at out-of-equilibrium demographic dynamic). I was thinking this may explain the large increase in virulence that you see on the first 300 days of figure 5B. (0 to 300 days is the period where the epidemic is expanding, figure 1).

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The authors have convincingly addressed most comments. Please correct the following points: L15 typo is still there L313 word missing (it the infectious bodies) L357 ”maximised for maximum CFR” sounds odd I do not need to see the paper again and wish to recommend it.

Evaluation round #1

DOI or URL of the preprint: **10.1101/108589**
Version of the preprint: 1

Authors' reply, 26 April 2017

Sofonea et al. present a theoretical study of EBOV's evolution. The high virulence of EBOV in the human host is thought to be maladaptive, because humans are not the main reservoir of the virus. As a consequence, EBOV's virulence is expected to decrease during long epidemics in humans, such as the outbreak that occurred in West Africa in 2013-2016. The present paper challenges this view and explores how, given EBOV's life cycle and known epidemiological parameters, virulence is expected to evolve in the human host during long epidemics. The main finding of the paper is that there is no chance that EBOV's virulence decreases in the short and long terms. In itself the idea that selection should select for higher virulence in diseases that are also transmitted after host death will sound intuitive for most evolutionary epidemiologists. The accomplishment of the paper is to make a very strong case that the parameter range where virulence could decrease is very small. The models show that EBOV's virulence is selected to be high even in human populations whatever the timescale considered. The models further provide scientifically grounded arguments in favor of the safe management of corpses. Altogether these results make a timely and important contribution to the knowledge and understanding of EBOV. For these reasons, we would be willing to write a recommendation for the paper. Before we do so, there are a number of issues that we would like the authors to address. Most of them are formal issues, but I attract your attention to Reviewer #2's suggestion about the short-term model and the structure of variance and covariances.

Thanks! **Reviewed by Virginie Ravigné**

L15: causes « one of » the most

Thanks! Sorry for the typo.

**L17: transmission routes, “including corpses”, we... **

We now write “three transmission routes (by regular contact, via corpses and by sexual contact)”

L20: replace adaptive reasons by “ultimate causes”. Previous hypotheses were non-adaptive or maladaptive.

L30: delete Indeed. And exchange the positions of the two ideas : first idea = previously it was believed that EBOV’s high virulence was due to adaption to the reservoir and subsequent maladaptation to human hosts; second idea = as a consequence one could expect that EBOV’s virulence would decrease with sustained circulation in human populations

Done.

L31: there is no question. Reformulate “to answer this question”

We now write “To address this problem”.

L32: routeS

L35: delete “unfortunately”

Done

L35: you are not able to demonstrate that EBOV’s virulence is not due to maladaptation. What you do, is provide another hypothesis. Then someone else may test the two hypotheses based on real data.

Indeed. We now write “Our results reveal that the virulence of EBOV might not be due to the maladaptation of the virus, but could rather originate from its unique life cycle.”

L43: please provide a short definition of CFR. How it relates to virulence can be stated later.

We added the precision “that is the ratio of infected hosts who die from the infection”

L47-53: The way the state-of-art is presented is surprising to say the least. There has been quite a huge amount of work between the 80’s beliefs and nowadays views on virulence evolution. There are better reasons than data on HIV or tuberculosis to discard the scenario of benignity, e.g., the whole literature on virulence evolution, optimal virulence, the trade-off hypothesis and so on… Please consider making a less biased presentation of the current understanding on virulence evolution.

Thank you for spotting our clumsy formulation. It was of course not our intention to say that the avirulence hypothesis is still dominant in evolution biology. Our writing was motivated by the fact that outside the field of evolutionary biology, especially in medicine or public health, many still consider that parasites must evolve toward benignity. We now clarify our opinion by distinguishing between evolutionary biology and public health.

L58: If figures are available, as e.g., the proportion of cases attributable to contamination by corpses, it could be useful to give them here.

We added the reference mentioned in Table 1 here.

L64-69: the big question should be given here… the paragraph supposed to focus the reader’s interest is a bit disappointing considering what the paper brings to the table. It is mere plan of further sections. The important point is to explore whether EBOV virulence is expected to decrease while the virus circulates in human populations – as believed from the maladaptation hypothesis - or not.

We now write: “Will EBOV become more virulent by adapting to humans? To address this question, we use mathematical modelling to determine how case fatality ratio affects the risk of emergence, how it evolves on the long and on the short term.”

L71: delete “in the long run”

L72: result (not results)

L72: delete “unique”

L90: delete ritual, and add “, mostly during ritual practices” after victims

Done

L102: Shouldn’t the value of gamma be computed only as the time between the onset of symptoms and death (not recovery) ?

Gamma is the rate at which the live infectious stage (I) ends. This is independent from the infection outcome (death or recovery), which is captured by the case fatality ratio. In the model, the time to recovery or death are identical but in reality, infections that clear could do so more (or less) rapidly than infections that lead to host

death. This is why we take the average of the two durations.

L120: body

Done.

L140-143: the two statements are contradictory. First you state that viruses evolve so rapidly that evolutionary and epidemiological timescales overlap, then you focus on evolution from standing variance. The idea of the first statement is precisely that mutations occur at the same timescale as population dynamics and can no longer be neglected in front of standing genetic variance. It is not very important but it would improve the manuscript to be more precise on the use of Price equation on one hand and adaptive dynamics on the other. For me, Price equation simply brings the possibility to consider the impact of genetic variance on short-term contemporary dynamics. And this is already very important and a sufficient reason to use this framework.

This is actually related to the main comment from reviewer #2 and his suggestion to add simulations where we would only vary virulence (see our response below for details). In the revised version, we clarified the pros and cons of adaptive dynamics vs. price equation models. We definitely agree that the two formalisms make contradictory assumptions, which is one of the motivations for presenting the results from both.

This brings me to a second remark. In the results section, AD results were presented first. Why not also in the methods. AD is a classical way to find out whether selection is stabilizing, directional or disruptive. It cannot really be sold as a way to study long term evolution (cf JEB 2005's special issue). It could be nice to put it first and to complete it with the Price part. And a small paragraph explaining the true complementarities of the two methods would nicely complete the end of the introduction.

We agree that having the same order in the Methods and the Results is a good idea and we also added a more careful comparison between the two approaches.

L151: delete "several"

Done

L154-170: Most of this should have been presented in Introduction

We of course hesitated to describe trade-offs in the Introduction but we decided to only include it in the Methods for 2 reasons. First, we wanted the introduction to be short and focused. Second, we find that our results are greatly independent of the underlying trade-offs. This is why we figured that introducing this notion early on might loose a general audience, without necessarily helping it to grasp the essence of the results.

Equ 2: Wouldn't it be reasonable to assume different trade-off shapes (p) for different transmission routes?

This is a difficult question to answer, especially since detailed data on transmission-virulence trade-off is not that abundant. In fact, this echoes one of the concerns about adaptive dynamics approach, which we briefly mention in the paper, which is that, as shown by critical function analyses, slight changes in trade-off shape are sufficient to have major consequences on evolutionary dynamics (not to mention of course epidemiological dynamics). One of the strong results of our study is that EBOV virulence evolution is largely independent of this trade-off shape and even to its existence. We now mention that p could vary amongst transmission routes.

L263-266 Unnecessary. Should have been clarified in the introduction.

Our decision to add these two sentences here was that, as for the trade-off notion, these details seemed unessential for a broad audience. Furthermore, beginning the section by "If we denote" seemed very dry.

L292 Virulence COULD be adaptive. Evidence is very indirect.

L295 delete therefore. Its is LIKELY exposed

Done

L305 One expects the big result (virulence is unlikely to decrease) here, with a few words about the intuitive explanation.

We now write "In addition to the strong selection for maximum CFR, another striking result"

L306-311 This paragraph comes a bit early.

We moved this paragraph after the next one (which helps with the sentence above).

L312 require (not requires)

L319 transitory

Done

L336-339 to be explained very early in the manuscript

As for the other points (trade-off and Price equation), we think this precision is for a narrow audience. We did hesitate to mention it in the Model section but thought that it would be more visible in the discussion.

L341 I see no mention of the need to measure the determinants of the evolutionary potential of the virus (mutation rate, distribution of mutation effects…), while it is crucial to predict evolution.

The substitution rate has been described in detail for this epidemics (we now mention it). The DFE is unlikely to be achievable for a human infectious disease as virulent as ebola virus. One of the alternatives, which we mention, consists in using the virus sequence to perform phylogenetic studies or GWAS studies (which we now mention). Finally, we took the liberty to also mention a very recent study that has shown in vitro that the ebola viruses from the 2014-2016 epidemics have acquired mutations allowing them to adapt to infecting human cells. **Reviewed by François Blanquart**

The authors analyse the evolution of virulence of Ebola virus. They convincingly and robustly demonstrate that in humans, Ebola optimal virulence is high, contrary to the hypothesis that Ebola may evolve lower virulence for better transmission. This is linked to the original life cycle of Ebola and in particular the fact that the virus can transmit from deceased individuals, thus reducing the cost of virulence.

Thanks!

The authors should elaborate the discussion of their model in the context of a disease that's not endemic to humans, spills over from the main host (the bat) and creates short-lived epidemics. This means the initial virus is not expected to be adapted to the human life cycle, and only has 1-2 years to adapt. The relevance of the models must be introduced in that context. In particular, the 'long-term' model may seem irrelevant if not well introduced.

We added a paragraph to specify that in the Price equation and in the adaptive dynamics, we here assume that the parasite can spread (R0) and therefore ignore the initial outbreak. To study such dynamics, an evolutionary rescue approach would be more appropriate due to the weight of the stochastic processes. We now make this explicit early in the Results section.

It is useful to present a short-term model, but the model could be simplified and better connected to the long-term model. All the covariances between traits make the model much more complex, these covariances do not seem to be linked specifically to short-term evolution, but rather to the formalism used, and the effects of all these covariances, and how these covariances evolve, are not detailed. The model could be simplified. For example, a simulation with 100 genotypes (with alpha = 0, 0.01, …, 0.99, 1), starting from a single genotype with a rather strong virulence, with mutation changing alpha (as in the adaptive dynamic model) to generate genetic variance. I feel this would be simpler, more in line with the adaptive dynamic model, and more biologically plausible than a model where genetic variation and covariations are imposed from the start.

We understand the concern but unfortunately there is no easy way to link the short-term and the long-term evolution model without running heavy simulations… To be more specific, in the long-term model, incorporating more than one trade-off relationship between parameters quickly generates dimensionality problems. This is why here we only consider a potential correlation between virulence (defined as case fatality ratio) and transmission rate. Another limitation is the time scales separation, i.e. we assume that epidemiological dynamics are at equilibrium. The short-term evolution model, by assuming that the genetic variance is fixed, allows us to incorporate more of these correlations between traits because the Price equation approach allows us to rule out unessential correlations. This allows to alleviate the two main assumptions of the long-term evolution model (time scales separation and multiple trade-offs/correlations). Unfortunately, no approach is perfect and the main limitation of the short-term evolution is that the variance/covariance matrixes are very

difficult to update. The only way to do so, would be to have explicit mutations, which means running detailed and complex numerical simulations that make strong assumptions too. Finally, about the suggestion to run a simulation model by varying only virulence, we are unsure what kind of information it would bring because its sole effect would be to check that the adaptive dynamics predictions are robust to strain diversity (i.e. having more than one resident strain). Since we assume no within-host interaction (no i.e. co- or superinfection) and only one susceptible host compartment, it is expected that – in your suggested one-trait evolution setting – one strain excludes all the others in the end, whatever the polymorphism through time. Therefore, may mutations occur randomly and overlap in time, or may they are all present at the beginning or sequentially replace each other, the long-term evolution of virulence is always governed by the selection gradient with respect to virulence. Hence we doubt of the existence of exclusive insights numerical transient polymorphism would provide compared to the classical AD approach. Given that the results we obtain are strong and very qualitative (compared for instance to studies that investigate evolutionary branching) and given that we assume no population structure that might affect mutant invasion dynamics (e.g. there is no spatial structure) we think these would unnecessarily complexify the manuscript. That being said, we did try to investigate trait evolution with a stochastic model where mutations occur at random. However the results appeared to be irrelevant because of numerical problems occurring due to the combination of the very low convergence speed to the epidemiological equilibrium which then causes a large amount of strains to coexist for long times and densities varying from very small (<1 for mutant strain infected hosts) to high levels (S>2E6). In our opinion, this would require a separate study a perhaps better suited model in order to alleviate these numerical issues.

Specific comments:

Abstract line 15, typo, should be "causes one of the most virulent"

Done

Introduction line 47-53: here one might one want to introduce the trade-off hypothesis - the idea that pathogens cannot optimise all their traits independently

Indeed! See reply to reviewer 1.

Line 51: 'intuitions can be misleading' is not really accurate, the theory may be wrong because it's not considering trade-offs between life-history traits

We removed the reference to "intuitions", which is indeed a very relative notion.

Somewhere around line 71-72 might want to be a bit more specific and give an intuition for the main result

To give an intuitive idea of the origin of the virulence, we added the following precision to describe the life cycle: "that includes transmission from hosts after death".

Throughout I would replace 'latency' with 'incubation' (latency suggests the virus is dormant and not replicating)

In epidemiology, the latent period is the time between the exposure of the host and the beginning of the infectious period. Incubation refers to the time between the exposure and the onset of the symptoms. Since transmission can occur before the onset of the symptoms (it's actually one of the key things to control an outbreak), we prefer to use latency.

**Line 147 I would specify 'dead bodies cease to be infectious' or 'corpses …' **

Done

Line 156: 'because without them predictions tend to be trivial' -> could be phrased differently, I think we incorporate trade-offs because it's more biologically realistic

We replaced "because" by "and" to remove the idea of causation.

Line 169-170 'setting up cohorts of sero-discordant people' -> 'identifying serodiscordant couples in cohorts'

Done

Line 215: somewhere in this paragraph it would be helpful to come back to equation (1) and write the contributions of the three components in your parameterisation.

The mention of equation 1 was indeed misleading. We now refer explicitly to the figure.

Line 219: 'the most virulent EBOV strains are always the most likely to emerge' -> this seems to contradict figure 2, absence of trade-off (p = 0, gray curve), safe burial (theta = 0), where R0 declines with virulence

Indeed, if p=\theta=0 R0 decreases with alpha, although given the default parameters this is difficult to see in Figure 2. We now specify that if there is no trade-off and no transmission from corpses, then R0 can decrease (slowly) with virulence.

Line 222: 'burial management can prevent emergence only if the transmission-virulence trade-off is strong enough': you may want to write 'is more likely if the transmission-virulence trade-off is strong enough'. If you had chosen lower bI, bC, b_D, the curves could have crossed the R0 = 1 line.

We now specify, "with our default parameter values".

Line 226: what is the 'lowest sustainable virulence'?

We now write "the lowest virulence that allows persistence"

Line 239-240 clarity could be improved, perhaps 'If the trade-off is weak, the CFR is weakly linked to transmission by regular contact and therefore selection on alpha only weakly depends on this component of the life cycle'

Thanks: we made the edit.

Line 244: explain why an interval, e.g. "It was not possible to find an explicit expression for the long-term equilibrium virulence, but we found it lies in the following interval:"

Done.

Line 283: when virulence correlates with transmission, you see further selection for virulence in the initial state of the epidemic? Perhaps link that with references (Bolker et al JRSI 2010, Berngruber Gandon et al Plos Path 2013). The converse effect, when virulence is correlated with incubation period, is interesting; but why was this particular correlation chosen, is it biologically plausible?

A correlation between virulence and transmission rate can indeed select for higher levels of virulence, which we mentioned in line 282 of the original manuscript. Our motivation to focus on the incubation period was double. First, the correlation involving transmission is now well described (Lenski & May 1994, Day & Proulx 2004, Day & Gandon 2007). Second, in the remainder of the text we show that our results can be obtained in absence of a transmission-virulence trade-off so it seemed logical to do the same for the Price equation. We now added the following precision when mentioning the correlation between transmission and CFR: which is consistent with earlier models \cite[e.g.]\DayProulx2004\ and studies \citep\BerngruberEtal2013\

Line 284 sentence incomplete

For clarity, we now write: A scenario where average virulence decreases initially is when it is positively correlated with the latency period

Line 336-341: the feedback between viral evolution (over one epidemic) and host evolution does not seem biologically plausible, given the short-time scale of an epidemic.

We apologize if the formulation was unclear: we did not mean genetic host evolution but rather a change in their immunological status. This can be seen as a coevolutionary interaction since the parasite population shapes the immunological status of the host population, which in returns shapes parasite evolution. We added the following sentence: Since the immunological status of the host population is determined by that of the virus population, this \textit\de facto\ qualifies as a coevolutionary interaction. Line 365: 'cadaver' -> perhaps use 'corpse' throughout? (also sometimes 'bodies' or 'dead bodies' is used). We now use dead bodies.

Below equation 8, some symbols are not defined. (the total population sizes). Also say that the system is not a closed form equation and that the covariances are themselves evolving.

The total population sizes do not seem to appear in equation 8. However, we agree it is worth mentioning here that the system is not closed (or at least that the correlations are assumed to be constant for simplicity). In the Methods, we write: An important assumption of this Price equation approach is that covariance terms are assumed to be constant, which implies that predictions are only valid on the short term.

New figure 4: areas are not colored. The legend suggests a bistable outcome between the dashed and solid line, is it the case?

About the colour, this is perhaps a conversion issue because on our side they seem coloured. The region that should appear in pink between the two lines is not bistable but has a positive selection gradient when alpha=0.5.

Table 1: sigma, should not be "post-mortem" but pre-mortem. Sup Mat, line 726 'neglect'

We now write "Elimination rate of convalescent hosts"

Sup Mat, line 726 'neglect'

Done

Decision by Virginie Ravigné, posted 03 April 2017

Revision needed

Sofonea et al. present a theoretical study of EBOV's evolution. The high virulence of EBOV in the human host is thought to be maladaptive, because humans are not the main reservoir of the virus. As a consequence, EBOV's virulence is expected to decrease during long epidemics in humans, such as the outbreak that occurred in West Africa in 2013-2016. The present paper challenges this view and explores how, given EBOV's life cycle and known epidemiological parameters, virulence is expected to evolve in the human host during long epidemics. The main finding of the paper is that there is no chance that EBOV's virulence decreases in the short and long terms. In itself the idea that selection should select for higher virulence in diseases that are also transmitted after host death will sound intuitive for most evolutionary epidemiologists. The accomplishment of the paper is to make a very strong case that the parameter range where virulence could decrease is very small. The models show that EBOV's virulence is selected to be high even in human populations whatever the timescale considered. The models further provide scientifically grounded arguments in favor of the safe management of corpses. Altogether these results make a timely and important contribution to the knowledge and understanding of EBOV. For these reasons, we would be willing to write a recommendation for the paper. Before we do so, there are a number of issues that we would like the authors to address. Most of them are formal issues, but I attract your attention to Reviewer #2's suggestion about the short-term model and the structure of variance and covariances.

Reviewed by Virginie Ravigné, 26 April 2017

I am fully convinced by the results of the paper. I mainly have presentation issues. In particular, for me, the Introduction section does a poor job in presenting the current understanding about the evolution of virulence, the main question addressed and the complementarity of the two approaches. Below are more specific comments that might be of some help to improve this very crucial section.

L15: causes « one of » the most

L17: transmission routes, "including corpses", we…

L20: replace adaptive reasons by "ultimate causes". Previous hypotheses were non-adaptive or maladaptive.

L30: delete Indeed. And exchange the positions of the two ideas : first idea = previously it was believed that EBOV's high virulence was due to adaption to the reservoir and subsequent maladaptation to human hosts; second idea = as a consequence one could expect that EBOV's virulence would decrease with sustained circulation in human populations

L31: there is no question. Reformulate "to answer this question"

L32: routeS

L35: delete "unfortunately"

L35: you are not able to demonstrate that EBOV's virulence is not due to maladaptation. What you do, is provide another hypothesis. Then someone else may test the two hypotheses based on real data.

L43: please provide a short definition of CFR. How it relates to virulence can be stated later.

L47-53: The way the state-of-art is presented is surprising to say the least. There has been quite a huge amount of work between the 80's beliefs and nowadays views on virulence evolution. There are better reasons than data on HIV or tuberculosis to discard the scenario of benignity, e.g., the whole literature on virulence evolution, optimal virulence, the trade-off hypothesis and so on... Please consider making a less biased presentation of the current understanding on virulence evolution.
L58: If figures are available, as e.g., the proportion of cases attributable to contamination by corpses, it could be useful to give them here.
L64-69: the big question should be given here... the paragraph supposed to focus the reader's interest is a bit disappointing considering what the paper brings to the table. It is mere plan of further sections. The important point is to explore whether EBOV virulence is expected to decrease while the virus circulates in human populations – as believed from the maladaptation hypothesis - or not.
L71: delete "in the long run"
L72: result (not results)
L72: delete "unique"
L90: delete ritual, and add ", mostly during ritual practices" after victims
L102: Shouldn't the value of gamma be computed only as the time between the onset of symptoms and death (not recovery) ?
L120: body
L140-143: the two statements are contradictory. First you state that viruses evolve so rapidly that evolutionary and epidemiological timescales overlap, then you focus on evolution from standing variance. The idea of the first statement is precisely that mutations occur at the same timescale as population dynamics and can no longer be neglected in front of standing genetic variance. It is not very important but it would improve the manuscript to be more precise on the use of Price equation on one hand and adaptive dynamics on the other. For me, Price equation simply brings the possibility to consider the impact of genetic variance on short-term contemporary dynamics. And this is already very important and a sufficient reason to use this framework. This brings me to a second remark. In the results section, AD results were presented first. Why not also in the methods. AD is a classical way to find out whether selection is stabilizing, directional or disruptive. It cannot really be sold as a way to study long term evolution (cf JEB 2005's special issue). It could be nice to put it first and to complete it with the Price part. And a small pargraph explaining the true complementarities of the two methods would nicely complete the end of the introduction.
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Equ 2: Wouldn't it be reasonable to assume different trade-off shapes (p) for different transmission routes?
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L295 delete therefore. Its is LIKELY exposed
L305 One expects the big result (virulence is unlikely to decrease) here, with a few words about the intuitive explanation.
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L341 I see no mention of the need to measure the determinants of the evolutionary potential of the virus (mutation rate, distribution of mutation effects...), while it is crucial to predict evolution.

Reviewed by **François Blanquart**, 26 April 2017

The authors analyse the evolution of virulence of Ebola virus. They convincingly and robustly demonstrate that in humans, Ebola optimal virulence is high, contrary to the hypothesis that Ebola may evolve lower virulence

for better transmission. This is linked to the original life cycle of Ebola and in particular the fact that the virus can transmit from deceased individuals, thus reducing the cost of virulence. The authors should elaborate the discussion of their model in the context of a disease that's not endemic to humans, spills over from the main host (the bat) and creates short-lived epidemics. This means the initial virus is not expected to be adapted to the human life cycle, and only has 1-2 years to adapt. The relevance of the models must be introduced in that context. In particular, the 'long-term' model may seem irrelevant if not well introduced. It is useful to present a short-term model, but the model could be simplified and better connected to the long-term model. All the covariances between traits make the model much more complex, these covariances do not seem to be linked specifically to short-term evolution, but rather to the formalism used, and the effects of all these covariances, and how these covariances evolve, are not detailed. The model could be simplified. For example, a simulation with 100 genotypes (with alpha = 0, 0.01, ..., 0.99, 1), starting from a single genotype with a rather strong virulence, with mutation changing alpha (as in the adaptive dynamic model) to generate genetic variance. I feel this would be simpler, more in line with the adaptive dynamic model, and more biologically plausible than a model where genetic variation and covariations are imposed from the start.

Specific comments:

Abstract line 15, typo, should be "causes one of the most virulent"

Introduction line 47-53: here one might one want to introduce the trade-off hypothesis - the idea that pathogens cannot optimise all their traits independently

Line 51: 'intuitions can be misleading' is not really accurate, the theory may be wrong because it's not considering trade-offs between life-history traits

Somewhere around line 71-72 might want to be a bit more specific and give an intuition for the main result

Throughout I would replace 'latency' with 'incubation' (latency suggests the virus is dormant and not replicating)

Line 147 I would specify 'dead bodies cease to be infectious' or 'corpses ...'

Line 156: 'because without them predictions tend to be trivial' -> could be phrased differently, I think we incorporate trade-offs because it's more biologically realistic

Line 169-170 'setting up cohorts of sero-discordant people' -> 'identifying serodiscordant couples in cohorts'

Line 215: somewhere in this paragraph it would be helpful to come back to equation (1) and write the contributions of the three components in your parameterisation.

Line 219: 'the most virulent EBOV strains are always the most likely to emerge' -> this seems to contradict figure 2, absence of trade-off (p = 0, gray curve), safe burial (theta = 0), where R0 declines with virulence

Line 222: 'burial management can prevent emergence only if the transmission-virulence trade-off is strong enough': you may want to write 'is more likely if the transmission-virulence trade-off is strong enough'. If you had chosen lower bI, bC, b_D, the curves could have crossed the R0 = 1 line.

Line 226: what is the 'lowest sustainable virulence'?

Line 239-240 clarity could be improved, perhaps 'If the trade-off is weak, the CFR is weakly linked to transmission by regular contact and therefore selection on alpha only weakly depends on this component of the life cycle'

Line 244: explain why an interval, e.g. "It was not possible to find an explicit expression for the long-term equilibrium virulence, but we found it lies in the following interval:"

Line 283: when virulence correlates with transmission, you see further selection for virulence in the initial state of the epidemic? Perhaps link that with references (Bolker et al JRSI 2010, Berngruber Gandon et al Plos Path 2013). The converse effect, when virulence is correlated with incubation period, is interesting; but why was this particular correlation chosen, is it biologically plausible?

Line 284 sentence incomplete

Line 336-341: the feedback between viral evolution (over one epidemic) and host evolution does not seem biologically plausible, given the short-time scale of an epidemic.

Line 365: 'cadaver' -> perhaps use 'corpse' throughout? (also sometimes 'bodies' or 'dead bodies' is used).

Below equation 8, some symbols are not defined. (the total population sizes). Also say that the system is

not a closed form equation and that the covariances are themselves evolving.

New figure 4: areas are not colored. The legend suggests a bistable outcome between the dashed and solid line, is it the case?

Table 1: sigma, should not be ”post-mortem” but pre-mortem. Sup Mat, line 726 ‘neglect’

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