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SHOULD WE AIM FOR GENETIC IMPROVEMENT IN HOST RESISTANCE OR TOLERANCE TO INFECTIOUS DISEASE?

Topic Editors
Andrea B. Doeschl-Wilson and
Ilias Kyriazakis

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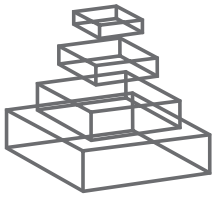

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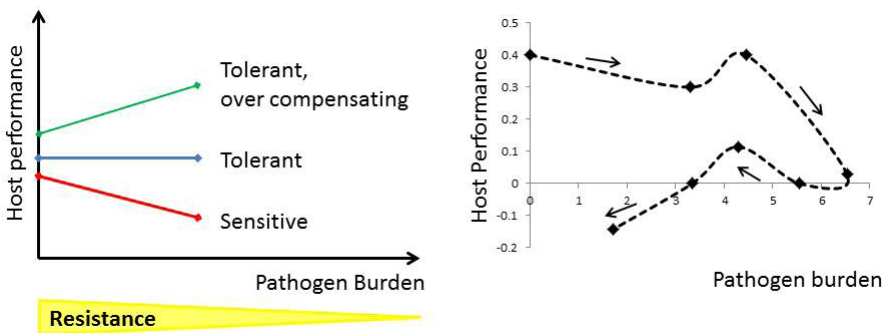
SHOULD WE AIM FOR GENETIC IMPROVEMENT IN HOST RESISTANCE OR TOLERANCE TO INFECTIOUS DISEASE?

Topic Editors:
Andrea B. Doeschl-Wilson, The Roslin Institute and R(D)SVS of the University of Edinburgh, United Kingdom
Ilias Kyriazakis, Newcastle University, United Kingdom



Recent advances in quantitative genetic and genomic studies have shed light on the important role of genetic control strategies for reducing disease risk and severity in livestock populations. There are two alternative host defence strategies to infectious pathogens that could be enhanced by genetic selection: improvement of host resistance versus improvement of host tolerance to infectious pathogens. Resistance refers to mechanisms that restrict the reproduction rate of a pathogen within a host, whilst tolerance mechanisms focus on minimising the damage that a pathogen inflicts on the host.

Both strategies may have a similar impact on individual host fitness and performance, but can have contrasting effects on population performance and disease risk and severity. For example, improving host resistance may result in successful eradication of a disease from a livestock population, whereas disease eradication may be difficult if hosts are tolerant as these can harbour the pathogen without showing obvious or severe symptoms. On the other hand, it has been argued that increasing host resistance would fuel the arms race between host and pathogen and stimulate pathogen evolution towards higher virulence. Increasing tolerance,

in contrast, imposes no or little selection pressure on the pathogen. Further, whereas disease resistance mechanisms may be specific to a particular pathogen (e.g. development of specific antibodies), tolerance mechanisms that repair damaged tissues are associated with the host rather than the pathogen, and are thus more likely to be generic to a range of pathogens. Hence, improving tolerance may be beneficial if individuals are exposed to a variety of pathogens or pathogen strains, and disease eradication has proven difficult.

In contrast to evolutionary biology and plant breeding, animal breeding has only recently started to seriously consider a distinction between disease resistance and tolerance and their consequences. However, a deeper understanding of the underlying mechanisms and implications of improving either or both of the host defence mechanisms on future disease risk and severity is urgently needed by animal scientists, veterinarians and breeders to make informed decisions that help to maintain healthy livestock populations and guarantee food security.

The topic 'genetic improvement of disease resistance v tolerance' would lend itself to research papers covering a variety of aspects that need to be considered, such as 'how to obtain genetic parameter estimates and genomic breeding values related to disease resistance / tolerance', 'evidence for host genetic influence of resistance or tolerance', 'genetic, genomic and immunological understanding of resistance / tolerance mechanisms', 'epidemiological consequences of improving disease resistance / tolerance'.

I believe that this research topic is both timely and relevant, and that sufficient knowledge is available across disciplines for composing valuable research / review articles that stimulate interest to a wide range of readers of Frontiers, and thus promote the growth of this journal.

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EDITORIAL
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Should we aim for genetic improvement in host resistance or tolerance to infectious pathogens?

***Andrea B. Doeschl-Wilson*[1]* and *Ilias Kyriazakis*[2]**

[1] *Genetics and Genomics, The Roslin Institute and R(D)SVS of the University of Edinburgh, Roslin, UK*
[2] *Department of Agriculture, Food, and Rural Development, Newcastle University, Newcastle upon Tyne, UK*
**Correspondence: andrea.wilson@roslin.ed.ac.uk*

Edited by:
Guilherme J. M. Rosa, University of Wisconsin, USA

Reviewed by:
Steve Bishop, University of Edinburgh, UK

There is an imperative to consider alternative strategies to pharmaceuticals to control infectious diseases amongst livestock. Recent advances in genetics and genomics have highlighted the potential for genetic control strategies to maintain high health and performance levels in livestock populations. This special research topic focuses on two alternative host defence strategies for coping with infectious pathogens that could be tackled for genetic improvement: **host resistance** vs. **host tolerance**. Resistance refers to mechanisms that restrict the reproduction rate of pathogens within a host, e.g., by blocking pathogen entry or limiting pathogen replication. Tolerance, in contrast, refers to the ability of a host to limit the detrimental impact pathogens can inflict on host performance (e.g., growth, milk/egg production, and fertility), without affecting pathogen burden *per se*. Tolerance captures all immune mechanisms that are not directly related to the reduction of pathogen burden, such as damage prevention or repair, as well as mechanisms that regulate self-harm inflicted by immune response components.

In contrast to the rapid expansion of identified genetic loci associated with host resistance, information of genetic loci or pathways associated with tolerance mechanisms is extremely sparse. However, a deeper understanding of the genetic control underlying both mechanisms is important in order to decide upon disease control strategies and avoid undesirable outcomes of genetic improvement programmes. This is firstly because at genetic level, resistance and tolerance may be antagonistically related. Secondly, although resistance and tolerance may have a similar impact on individual health and performance, they can have contrasting effects on performance outcomes and on disease prevalence at a population level. In particular, whilst improving host resistance could lead to disease eradication, this is unlikely if hosts are tolerant, as they can harbor the pathogen without showing obvious symptoms. On the other hand, it has been argued that increasing host resistance (but not tolerance) would fuel the arms race between host and pathogen, and stimulate pathogen evolution toward higher virulence. Furthermore, whereas disease resistance mechanisms are often pathogen-specific (e.g., mobilization of specific immune cells), tolerance mechanisms that prevent or repair damage may be more host than pathogen specific, and may thus offer generic protection for a range of pathogens. Hence, changing tolerance may be more beneficial in situations where individuals are exposed to a variety of pathogens or pathogen strains (as is the case in many commercial farms), with high risk of pathogen evolution, and where disease eradication has proven difficult (e.g., if asymptomatic carriers are present).

In contrast to evolutionary biology and plant breeding, livestock breeding has only recently started to appreciate the importance of distinguishing between resistance and tolerance to pathogens and to study their relationship and implications. This special research topic draws together animal scientists with expertise in molecular and quantitative genetics, immunology, epidemiology, evolutionary biology, and mathematical modeling to address the question "Should we aim for genetic improvement of host resistance or host tolerance to infectious pathogens" from different perspectives. The diverse contributions to this topic:

1. Provide an overview of the state-of-the-art understanding of resistance and tolerance in domestic livestock populations, with a focus on the application of genetic and genomic tools for host genetic improvement of either.
2. Lay out methodologies and data requirements for accurately quantifying resistance and tolerance for subsequent genetic studies.
3. Investigate the advantages and disadvantages of improving resistance vs. tolerance for specific relevant livestock diseases.

This special research topic kicks off with our own contribution that sets the stage for the development of the topic by the other authors (Doeschl-Wilson et al., 2012a,b). Hypothesizing that genetic improvement of host tolerance to infectious pathogens is first of all handicapped by difficulties in determining the tolerance phenotype, we then investigate what is needed to obtain accurate estimates of tolerance phenotypes. Our first article concentrates on group tolerance, which is the current state-of-the-art for quantifying tolerance, whereas the second article proposes new analytical solutions for extending the framework to the level of individuals. Complementary to this, Kause and Ødegård (2012) present recent statistical methods to estimate genetic parameters associated with tolerance and tolerance-related traits. Each method requires careful consideration of data requirements and underlying conditions for implementation into future breeding programmes.

Glass (2012) addresses resistance and tolerance from an immunological perspective at molecular level. Reviewing sequential immunological processes involved in the host response

to infectious pathogens, she explores whether resistance and tolerance mechanisms are likely to be controlled by the same set of genes or molecular pathways, and proposes new avenues for identifying new resistance or tolerance genes. As resistance and tolerance constitute two alternative, resource-costly, host defence mechanisms, a trade-off between both strategies may occur when resources are limited. Rauw (2012) investigates origins and consequences of such trade-offs by considering resistance and tolerance from a resource allocation viewpoint.

As emphasized in this special topic and elsewhere, the relative merits of improving host resistance and tolerance require careful consideration and may differ between diseases and livestock populations. Looking at the wider implications of improving either trait, Bishop (2012) addresses under what circumstances tolerance may be worth considering as a breeding goal, and applies his theoretical framework to nematode infections in ruminants. Guy et al. (2012) review current perspectives on selective breeding for disease resistance and tolerance in pigs, with an emphasis on industry applications. Rowland et al. (2012) discuss recent evidence of host genetic control in the response of pigs to the Porcine Reproductive and Respiratory Syndrome (PRRS) virus, in the light of resistance and tolerance. PRRS is a high priority pig disease world-wide, and currently there is a lively debate on whether to improve resistance or tolerance. Looking at Salmonella and Campylobacter infections in poultry, Calenge and Beaumont (2012) present an example where host tolerance to infectious pathogens is undesirable, and review current knowledge about host genetic control of two distinct resistance mechanisms, i.e., resistance to intestinal colonization and resistance to bacterial persistence. Finally, Detilleux (2012) uses a mathematical modeling approach to investigate the implications of selection for improved resistance or tolerance on performance and health at the level of individuals and populations, when applied to bovine mastitis. Mathematical models have the advantage that traits which are difficult to measure in practice, such as tolerance, can be predicted. This enables the role of influencing factors to be assessed systematically.

We hope that this special research topic moves us a step forward in our understanding of these two important, highly complex traits associated with livestock health and production, and the development of sustainable genetic improvement strategies.

REFERENCES

Bishop, S. (2012). A consideration of resistance and tolerance for ruminant nematode infections. *Front. Gene.* 3:168. doi: 10.3389/fgene.2012.00168

Calenge, F., and Beaumont, C. (2012). Toward integrative genomics study of genetic resistance to Salmonella and Campylobacter intestinal colonisation in fowl. *Front. Gene.* 3:261. doi: 10.3389/fgene.2012.00261

Detilleux, J. (2012). A mathematical model to study the resistance and tolerance to infection at the animal and population levels. *Front. Gene.* 3:146. doi: 10.3389/fgene.2012.00146

Doeschl-Wilson, A. B., Bishop, S. C., Kyriazakis, I., and Villanueva, B. (2012a). Novel methods for quantifying individual host response to infectious pathogens for genetic analyses. *Front. Gene.* 3:266. doi: 10.3389/fgene.2012.00266

Doeschl-Wilson, A. B., Villanueva, B., and Kyriazakis, I. (2012b). The first step toward genetic selection for host tolerance to infectious pathogens: obtaining the tolerance phenotype through group estimates. *Front. Gene.* 3:265. doi: 10.3389/fgene.2012.00265

Glass, E. J. (2012). The molecular pathways underlying host resistance and tolerance to pathogens. *Front. Gene.* 3:263. doi: 10.3389/fgene.2012.00263

Guy, S. Z. Y., Thomson, P. C., and Hermesch, S. (2012). Selection of pigs for improved coping with health and environmental challenges: breeding for resistance or tolerance? *Front. Gene.* 3:281. doi: 10.3389/fgene.2012.00281

Kause, A., and Ødegård, J. (2012). The genetic analysis of tolerance to infections: a review. *Front. Gene.* 3:262. doi: 10.3389/fgene.2012.00262

Rauw, W. M. (2012). Immune response from a resource allocation perspective. *Front. Gene.* 3:267. doi: 10.3389/fgene.2012.00267

Rowland, R., Lunney, J., and Dekkers, J. (2012). Control of porcine reproductive and respiratory syndrome (PRRS) through genetic improvements in disease resistance and tolerance. *Front. Gene.* 3:260. doi: 10.3389/fgene.2012.00260

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The first step toward genetic selection for host tolerance to infectious pathogens: obtaining the tolerance phenotype through group estimates

Andrea B. Doeschl-Wilson[1]*, Beatriz Villanueva[2] and Ilias Kyriazakis[3]

[1] *The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, UK*
[2] *Departamento de Mejora Genética Animal, INIA, Madrid, Spain*
[3] *School of Agriculture, Food and Rural Development, Newcastle University, Newcastle upon Tyne, UK*

Edited by:
Guilherme J. M. Rosa, University of Wisconsin-Madison, USA

Reviewed by:
Scott Newman, Genus plc, USA
Andrea Graham, Princeton University, USA

****Correspondence:***
Andrea B. Doeschl-Wilson, The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Midlothian EH26 9RG, UK.
e-mail: andrea.wilson@roslin.ed.ac.uk

Reliable phenotypes are paramount for meaningful quantification of genetic variation and for estimating individual breeding values on which genetic selection is based. In this paper, we assert that genetic improvement of host tolerance to disease, although desirable, may be first of all handicapped by the ability to obtain unbiased tolerance estimates at a phenotypic level. In contrast to resistance, which can be inferred by appropriate measures of within host pathogen burden, tolerance is more difficult to quantify as it refers to change in performance with respect to changes in pathogen burden. For this reason, tolerance phenotypes have only been specified at the level of a group of individuals, where such phenotypes can be estimated using regression analysis. However, few stsudies have raised the potential bias in these estimates resulting from confounding effects between resistance and tolerance. Using a simulation approach, we demonstrate (i) how these group tolerance estimates depend on within group variation and co-variation in resistance, tolerance, and vigor (performance in a pathogen free environment); and (ii) how tolerance estimates are affected by changes in pathogen virulence over the time course of infection and by the timing of measurements. We found that in order to obtain reliable group tolerance estimates, it is important to account for individual variation in vigor, if present, and that all individuals are at the same stage of infection when measurements are taken. The latter requirement makes estimation of tolerance based on cross-sectional field data challenging, as individuals become infected at different time points and the individual onset of infection is unknown. Repeated individual measurements of within host pathogen burden and performance would not only be valuable for inferring the infection status of individuals in field conditions, but would also provide tolerance estimates that capture the entire time course of infection.

Keywords: tolerance, resistance, phenotype, infectious disease, livestock, genetic selection

INTRODUCTION

Improvement of host responses to infectious challenges by genetic means is now widely recognized to be a valuable complement to conventional disease control in livestock. Disease traits have been difficult to target by traditional selection, but recent developments in high throughput genomics provide opportunities to dissect host responses to infectious pathogens and to increase the accuracy of selection. Resistance and tolerance are two distinct mechanisms of host response to infectious pathogens that could be targeted for genetic improvement. Resistance refers to the host ability to reduce pathogen invasion or replication, whereas tolerance refers to the host ability to maintain performance and fitness counteracting thus the damage that pathogens can inflict on it. Consequently, resistance is typically described as an inverse measure of pathogen burden (Råberg et al., 2007), whilst tolerance is described in terms of change of host performance or fitness as a result of change in pathogen burden (e.g., Simms, 2000).

Genetic analyses of disease data focus mainly on resistance mechanisms. State-of the art methods in genetic analysis of resistance of livestock to infectious disease have been discussed and outstanding challenges for obtaining reliable estimates of resistance parameters have been highlighted (e.g., Morris, 2007; Bishop and Woolliams, 2010; Ødegård et al., 2011; Bishop et al., 2012). Tolerance mechanisms as a host defense strategy have been extensively studied in plant species (Caldwell et al., 1958; Schafer, 1971). In animals, awareness of the important role of tolerance is rapidly increasing in immunology and evolutionary ecology (Råberg et al., 2007, 2009; Read et al., 2008; Ayres and Schneider, 2012; Medszhitov et al., 2012). However, in the context of livestock breeding, where "breeding for disease resistance" has attracted a significant research effort, it appears that very little attention has been paid to the "breeding for increased tolerance."

The lack of attention to tolerance of livestock to infectious pathogens in the scientific literature is surprising, given the increasing need to make livestock production more efficient and

sustainable in the face of challenges arising from the demands on global food production and climate change (Foresight annual review, 2011). Given that pathogen challenges are ubiquitous and manifold, "maintaining performance in the face of infectious challenge" or "reducing the impact of pathogens on performance" (i.e., the very definition of tolerance), appears to be a valuable breeding goal, at least at first instance. Also, both theoretical and empirical evidence suggest that a trade-off between resistance and tolerance may exist (e.g., Simms and Triplett, 1994; Mauricio et al., 1997; Pilson, 2000). This would imply that attempts to control infectious disease in a population by improving host resistance without considering the consequences on performance may fail if resistance and tolerance are antagonistically related (Doeschl-Wilson et al., 2009a,b).

There are several potential reasons why improvement of host tolerance to pathogens has received relatively little attention in livestock breeding. Some of these reasons are outlined below and constitute the first part of this paper. A close examination of these lead us to hypothesize that genetic improvement of host tolerance to infectious pathogens may be first of all handicapped by our ability to obtain reliable estimates of tolerance at a phenotypic level. Therefore, the aim of this article and its companion paper (Doeschl-Wilson et al., 2012) is to establish what measurements are needed to obtain accurate phenotypic tolerance estimates for genetic studies, and which factors need to be considered in the statistical analyses involved in such studies. Here, generic theoretical concepts for obtaining tolerance phenotypes are presented and their implementations for estimating tolerance for a group of individuals are discussed. In the companion paper we address the question whether tolerance can also be estimated at the level of individuals.

WHY HAS GENETIC IMPROVEMENT OF TOLERANCE RECEIVED LITTLE ATTENTION IN LIVESTOCK GENETIC RESEARCH?

There are at least four potential reasons for the apparent scarcity on host tolerance in livestock in genetic research.

THE IMPORTANCE OF DISTINGUISHING BETWEEN RESISTANCE AND TOLERANCE IN THE ANIMAL BREEDING CONTEXT HAS NOT BEEN BROUGHT TO ATTENTION

The ambiguity and frequent misuse of the terminology when referring to disease traits would support this hypothesis. Whilst "breeding for disease resistance" has become a well-established term, closer inspection reveals that it is not always clear whether the disease trait under consideration refers to resistance rather than to tolerance. For example, infection-induced mortality is a trait commonly used when describing disease resistance in farm species, particularly in fish (Houston et al., 2010; Ødegård et al., 2011). Mortality could actually refer to host resistance, where the animal dies because it cannot control pathogen replication, although the actual damage inflicted by a unit of pathogens may be low. On the other hand, mortality could also refer to tolerance, where the animal dies as a result of much damage inflicted by a unit of pathogens, although the actual pathogen burden may be low.

Another example where resistance and tolerance are frequently confused or used interchangeably, is when dealing with the trypano-tolerance of ruminants, which often refers to disease resistance mechanisms (Naessens, 2006) or is used to encompass both resistance and tolerance traits (Kemp and Teale, 1998). For example, Kemp and Teale (1998) state that "trypano-tolerant cattle show a remarkable *resistance* to the effects of African trypanosomiasis: they can tolerate the presence of parasites while apparently controlling levels of parasitaemia and, crucially not showing the severe anemia and production loss that are characteristics of infection in susceptible hosts."

Both host resistance and tolerance enhance host fitness, but distinguishing between these mechanisms is critical in genetic improvement programs, not only because they may be to be antagonistically related (Simms and Triplett, 1994; Fineblum and Rausher, 1995; Tiffin, 2000; Blanchet et al., 2010), but also because they can lead to strikingly different epidemiological and evolutionary outcomes, as outlined below.

GENETIC IMPROVEMENT OF RESISTANCE IS CONSIDERED FAVORABLE OVER GENETIC IMPROVEMENT OF TOLERANCE

Genetic improvement of host resistance as a disease control strategy may be thought favorable over improving tolerance due to their different epidemiological and evolutionary consequences. For instance, disease eradication in a population can only be achieved through increasing resistance, as improving tolerance does not constrain pathogen replication (Roy and Kirchner, 2000). Epidemiological theory further suggests that a threshold density of susceptible hosts is needed for an infection to spread effectively in a population (Keeling and Rohani, 2008). Thus, genetic selection may strive toward generating a sufficiently large proportion of resistant individuals to prevent epidemic outbreaks (MacKenzie and Bishop, 1999). Genetic selection for pathogen resistance has indeed led to reduced disease prevalence in farm species, as exemplified in the case of scrapie in sheep (Baylis et al., 2004), *Escherichia coli* F18 infections in pigs (Meijerink et al., 1997 and intestinal pancreatic necrosis in salmon Ødegård et al., 2009; Houston et al., 2010). However, evolutionary theory suggests that genetic selection for disease resistance may increase pathogen virulence, which should not occur when selecting for tolerance (Roy and Kirchner, 2000). This host-pathogen coevolution may counteract the short-term benefits of genetic selection on animal health, as demonstrated in the case of Mareks disease in poultry (Zelnik, 2003), where selection has only led to short-term reduction in disease prevalence. Indeed, it has been argued that increases in tolerance by selective breeding may be more evolution-proof than manipulations in resistance, because tolerance does not impose selection for pathogen counter-measures (Rausher, 2001).

Genetic improvement of host tolerance may thus be desirable in cases where overall host resistance is low leading thus to high infection prevalence in the population and low chance of elimination of the infection from the population, as is the case for nematode infections or mastitis in ruminants (Bishop, 2012). In fact, a recent simulation study modeling mastitis in dairy cattle (Detilleux, 2011) suggested that under certain conditions increasing individual tolerance could be more effective

for maintaining population health and performance than increasing individual resistance. Accumulated theoretical and empirical evidence would thus suggest that it is not *a priori* evident whether selection for host resistance is favorable over selection for host tolerance or *vice versa*. The answer is likely to be case specific and will depend on of both host and pathogen properties.

GENETIC SELECTION FOR IMPROVED HOST TOLERANCE IS NOT POSSIBLE DUE TO LACK OF GENETIC VARIATION

The existence of genetic variation (heritability) for the trait under consideration is a fundamental requirement for achieving genetic improvement through selection. Evolutionary arguments suggest greater genetic variation in host resistance than in tolerance. For instance, Read et al. (2008) indicated that "the scientific focus on resistance may be because parasite killing mechanisms are more likely to be genetically variable because of host–parasite coevolution." Furthermore, Roy and Kirchner (2000) argued on theoretical grounds that a tolerance gene should be more likely to be driven to fixation by natural selection than a resistance gene, and supported their theoretical concept with a number of examples across diverse plant species where resistance genes tended to be polymorphic and tolerance genes tended to be fixed. The theory has been supported in animal species; for example, a recent study identified genetic variation in resistance, but not in tolerance of monarch butterflies to a protozoan parasite (Lefèvre et al., 2011).

However, numerous empirical studies in a variety of plant and animal species provide evidence to the contrary (Simms and Triplett, 1994; Fineblum and Rausher, 1995; Mauricio et al., 1997; Koskela et al., 2002; Råberg et al., 2007; Blanchet et al., 2010) and would suggest that genetic variation in tolerance is actually a common phenomenon. For this reason, several theoretical arguments have been put forward to reconcile the apparently contradictory empirical findings with existing theory. These include genetic trade-offs between host fitness in pathogen free environments and tolerance (Agrawal et al., 1999; Tiffin and Rausher, 1999), or tolerance mechanisms acting on fecundity rather than on host survival (Best et al., 2008), as potential mechanisms responsible for maintaining genetic variation in tolerance. These arguments support the existence of genetic variation in host tolerance in animal species.

OBTAINING RELIABLE TOLERANCE PHENOTYPES FOR GENETIC ANALYSES IS CHALLENGING

Resistance and tolerance cannot be measured directly but need to be inferred from more readily available measures of other traits. As resistance refers to mechanisms that reduce pathogen invasion or replication within a host it is typically defined as the inverse of within host pathogen burden (number or mass of parasites per host or per unit host tissue) (Simms and Triplett, 1994; Råberg et al., 2007; Kause, 2011). Tolerance, on the other hand, is defined as the rate of change in host fitness with regards to changes in pathogen burden, and as such is consistent with the definition of the slope when regressing fitness against pathogen burden (Simms and Triplett, 1994; Simms, 2000; Råberg et al., 2009; Kause, 2011).

The concept of tolerance originates from evolutionary ecology and thus the generic term "fitness" has been widely used as response variable for describing tolerance. In animal science, depending on the type of disease, species and breeding goal, the most appropriate choice of response variable may be a fitness related trait (e.g., reproduction or survival trait), but also a measurable production trait. From now on we will use the term performance as a generic term for the response variable when defining tolerance.

The concept of tolerance is simple: a slope value of zero refers to complete tolerance, negative slopes to incomplete tolerance where host performance is reduced due to pathogens, and positive slopes to a mutualistic relationship between host fitness and the pathogens (sometimes called overcompensation). In case of incomplete tolerance, the steeper the slope, the lower the tolerance. However, as outlined in detail below, obtaining accurate phenotypes for this trait is challenging, partly because tolerance refers to a rate of change of a measurable quantity rather than to the quantity itself. We consider the difficulties entailed in estimating tolerance to be the main bottleneck why breeding for tolerance in livestock has received little attention. For this reason specifying the tolerance phenotype, both at theoretical and practical level, constitutes the main focus of our paper.

THEORETICAL CONSIDERATIONS WHEN SPECIFYING THE TOLERANCE PHENOTYPE

SPECIFYING PATHOGEN BURDEN

The need to measure pathogen burden when quantifying tolerance

Numerous studies have investigated the impact of infection on performance (e.g., Van der Waaij et al., 2000; Vagenas et al., 2007; Doeschl-Wilson et al., 2008, 2009a; Lewis et al., 2009) and compared the performance of animals in non-infectious and infectious environments without quantifying the actual pathogen burden (e.g., Mackinnon et al., 1991; Bisset and Morris, 1996; Naessens, 2006; Doeschl-Wilson et al., 2009b). Do such studies provide useful information on host tolerance?

The ability of animals to maintain relatively undiminished performance levels whilst infected is usually called *resilience* (Albers and Gray, 1986; Bisset and Morris, 1996). Thus resilience and tolerance are both concerned with the impact of infection on performance. However, whereas the definition of tolerance as a rate of change in performance due to changes in pathogen burden implies that tolerance cannot be inferred without quantifying pathogen burden, resilience studies usually do not include a measure of pathogen burden. Instead variation in performance is assessed in relation to an unknown standard level of pathogen challenge to which all individuals are assumed to be equally exposed (Bisset and Morris, 1996). As a consequence of this resilience conflates resistance and tolerance.

To illustrate this, consider the example illustrated in **Figure 1A** for two individuals exposed to the same environmental pathogen burden (or challenge dose). The individuals are assumed to differ in their resistance to the pathogen in question (**Figure 1A**) and (for ease of illustration) have different constant growth rates in

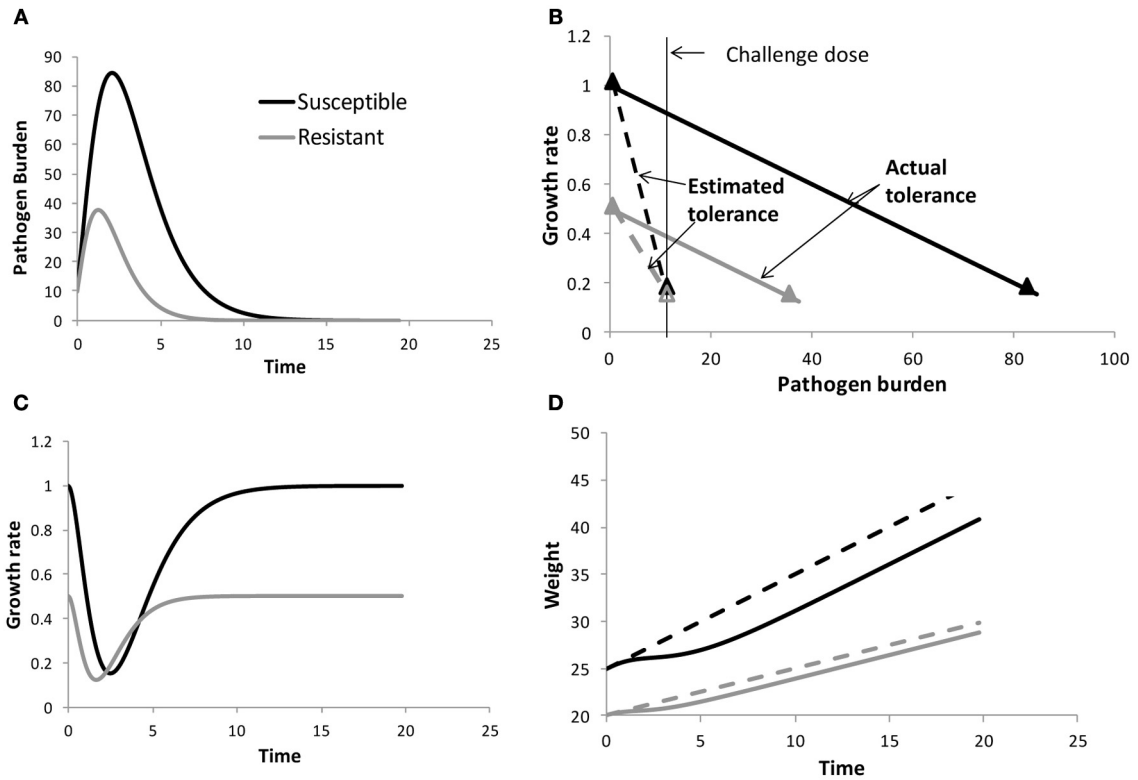

FIGURE 1 | Schematical figure to demonstrate the importance of measuring within host pathogen burden when estimating tolerance. The panels show pathogen burden and performance profiles for two individuals differing in resistance (panels **A**), but having the same tolerance (as indicated by the slope of the solid lines in panels **B**). Here the performance trait growth rate y was assumed to depend linearly on pathogen burden PB, i.e., $y(t) = y0(t) - bPB(t)$, where $y0$ refers to growth rate corresponding to $PB = 0$ and b is the tolerance slope. For ease of illustration $y0$ was assumed to differ between the individuals. The slopes of the dashed lines in panels **(B)** refer to the estimated tolerance when ignoring within host pathogen burden. panels **(C)** and **(D)** show the resulting growth rate and body weight time profiles, respectively. The dashed lines in panels **(D)** refer to the body weight profiles of both individuals in the absence of pathogen challenge. Information on how the data were generated can be found in the Appendix.

the absence of pathogen challenge, but have the same tolerance (i.e., same reduction in growth rate with increasing pathogen burden, **Figure 1B**). Due to differences in resistance, the pathogen replicates at different rates within both hosts, and as a consequence the susceptible individual experiences a greater reduction in growth rate and thus also in body weight over time than the resistant individual (**Figures 1C,D**). Thus, comparison of performance profiles alone (**Figure 1D**) may reveal differences in resilience, but does not provide information on tolerance. Taking pathogen burden into account is crucial for avoiding confounding effects between resistance and tolerance. Moreover, only by considering pathogen burden explicitly can we answer the crucial question of how performance would be affected by changes in pathogen challenge (e.g., caused by epidemic outbreaks or by genetic selection for improved host resistance or tolerance).

The need to use within-host pathogen burden rather than environmental pathogen burden or challenge dose when quantifying tolerance

Having established that pathogen burden needs to be taken into account when measuring tolerance, the next question is how to quantify it. Given that the study of tolerance originates from ecology, where tolerance analyses follow the methodology of reaction norms (Via and Lande, 1985; Simms, 2000), i.e., the pattern of phenotypes produced by a given genotype under different environmental conditions, it may seem natural to consider pathogen burden as an environmental rather than a host characteristic.

The definition of pathogen burden as an environmental characteristic may be attractive from a practical point of view. For instance, tolerance could be obtained as the slope of performance measured in a breeding nucleus with generally low pathogen burden compared with performance in a more pathogenic commercial environment, using estimates of environmental pathogen burden in either environment. Similarly, immunologists who think of tolerance as a dose response curve (Ayres and Schneider, 2012), may define pathogen burden by the challenge dose in an infection experiment (e.g., Lefèvre et al., 2011). Both types of definitions (i.e., environmental pathogen burden or inoculation dose) thus assume that the independent variable pathogen burden is the same for all individuals and constant over time. Although attractive for practical reasons, using environmental burden or inoculation dose could however lead to biased estimates of individual tolerance due to confounding effects between resistance

and tolerance. This is illustrated in **Figure 1** for two individuals having the same tolerance, but differing in resistance. Although both individuals are initially challenged with the same pathogen burden, within host pathogen burden will eventually differ due to differences in host resistance (**Figure 1A**). At any given time post infection, the susceptible host will have greater loss in performance than the resistant host due to greater within host pathogen burden. If these differences in pathogen burden are not taken into consideration, and within host burden was replaced by a constant environmental or challenge burden in the performance *vs.* pathogen burden plot, the resulting tolerance slope would be affected. In the illustrated example (**Figure 1B**), the slope of the susceptible individual would become much steeper than the slope of the resistant individual, suggesting differences in tolerance despite both individuals having equal tolerance. This simple example demonstrates that quantifying tolerance requires measuring individual within-host pathogen load rather than environmental burden or challenge dose in order to avoid confounding effects between resistance and tolerance and to obtain thus unbiased tolerance estimates.

THE NEED TO ACCOUNT FOR INDIVIDUAL VARIATION IN PERFORMANCE IN THE ABSENCE OF PATHOGEN CHALLENGE WHEN QUANTIFYING TOLERANCE OF A GROUP

The definition of tolerance as a slope stipulates that multiple measurements of performance related to different levels of pathogen burden are required. This requirement has led several researchers to conclude that tolerance can only be determined at the level of groups of individuals (e.g., family, breed, or line) (Mauricio et al., 1997; Råberg et al., 2007, 2009). In fact, to the best of our knowledge, all quantitative genetic analyses of tolerance to date have specified tolerance at the level of the group rather than the individual (McIntyre and Amend, 1978; Simms and Triplett, 1994; Mauricio et al., 1997; Pilson, 2000; Kover and Schaal, 2002; Råberg et al., 2007; Blanchet et al., 2010; Lefèvre et al., 2011). In these analyses the group specific tolerance estimate is usually obtained by regressing the performance of individual group members against their respective pathogen burden recorded at a specific point in time.

However, even in the case of a simple linear relationship between host performance y and pathogen burden PB for individual i of group j (as it is assumed in the majority of studies), i.e.,

$$y_{ij} = y0_{ij} + b_{ij}PB_{ij} \quad (1)$$

there are three sources of variation between individual group members: (i) resistance, represented by heterogeneous values for PB_{ij}, (ii) tolerance, represented by heterogeneity in the slopes b_{ij}, and (iii) vigor, i.e., individual performance in the absence of pathogen challenge, represented by heterogeneity in the intercepts $y0_{ij}$ (Stowe et al., 2000). Moreover, the three traits may be correlated, representing for example, trade-offs between resistance, tolerance and vigor (Mauricio et al., 1997; Agrawal et al., 1999; Pilson, 2000; Doeschl-Wilson et al., 2008). As illustrated in **Figure 2**, within group variation and co-variation between these traits can have a profound impact on the performance vs. pathogen burden relationship, and thus on group specific tolerance estimates. The figure depicts performance vs. within host pathogen burden for two families consisting of five individuals each. For ease of illustration it was assumed that families have the same average tolerance ($\bar{b} = -0.01$) and the same average vigor, but differ in average resistance. It was assumed that there is (the same) within family variation in all three traits, i.e., resistance (PB), vigor ($y0$), and tolerance (b). The difference between the top and bottom panels of **Figure 2** is that traits are either independent (**Figures 2A,B**) or highly correlated (**Figures 2C,D**).

Figures 2A,C show that simply regressing performance against pathogen burden would lead to poor estimates of family tolerance. Not only do the regression slopes differ substantially between both families, but in some cases family tolerance slope estimates are even positive suggesting overcompensation ($b > 0$) rather than incomplete tolerance ($b < 0$). The accuracy of family tolerance estimates improves substantially after adjusting performance for individual variation in vigor (i.e., using $y_{ij,adj} = y_{ij} - y0_{ij}$ or including $y0_{ij}$ as a covariate in the regression analysis), as shown in **Figures 2B,D**.

The results of this simple simulation would thus suggest that estimating group tolerance not only requires information of individual pathogen burden and performance post infection, but also information about individual vigor. A recent simulation study has demonstrated that accounting for individual variation in vigor is not only necessary for obtaining reliable phenotypic tolerance estimates, but also for obtaining unbiased estimates of genetic parameters associated with this trait (Kause, 2011).

THE INFLUENCE OF HOST-INDUCED CHANGE IN PATHOGEN VIRULENCE ON THE TOLERANCE PHENOTYPE OF A GROUP

One important aspect that has been ignored in the definitions and approaches outlined so far is that the impact of the pathogens on host performance (here defined as pathogen virulence) may change over the time course of infection. As outlined by Ayres and Schneider (2012) changes in such pathogen virulence are likely to arise from interactions between host immune response and the pathogen, and this makes host tolerance and pathogen virulence practically inseparable. Indeed, a host may be considered to be defined as tolerant merely because it reduces pathogen virulence over time without altering the pathogen burden *per se*. For this reason it has been proposed to consider host-induced change in pathogen virulence as a tolerance effect (Little et al., 2010; Ayres and Schneider, 2012). But how does this additional component of tolerance affect the phenotypic tolerance estimates of a group of individuals?

Host-induced changes in pathogen virulence may be represented by extending the original model (1) relating the performance of an individual i from family j at time t to its pathogen burden as follows:

$$y_{ij}(t) = y0_{ij}(t) + k_{ij}(t)PB_{ij}(t) \quad (2)$$

where $k_{ij}(t)$ is the time-dependent tolerance slope equal to $b_{ij} - v_{ij}(t)$, where b_{ij} refers to tolerance in the original sense (i.e., change in performance due to change in pathogen burden) and $v_{ij}(t)$ refers to the rate at which the pathogen's virulence changes

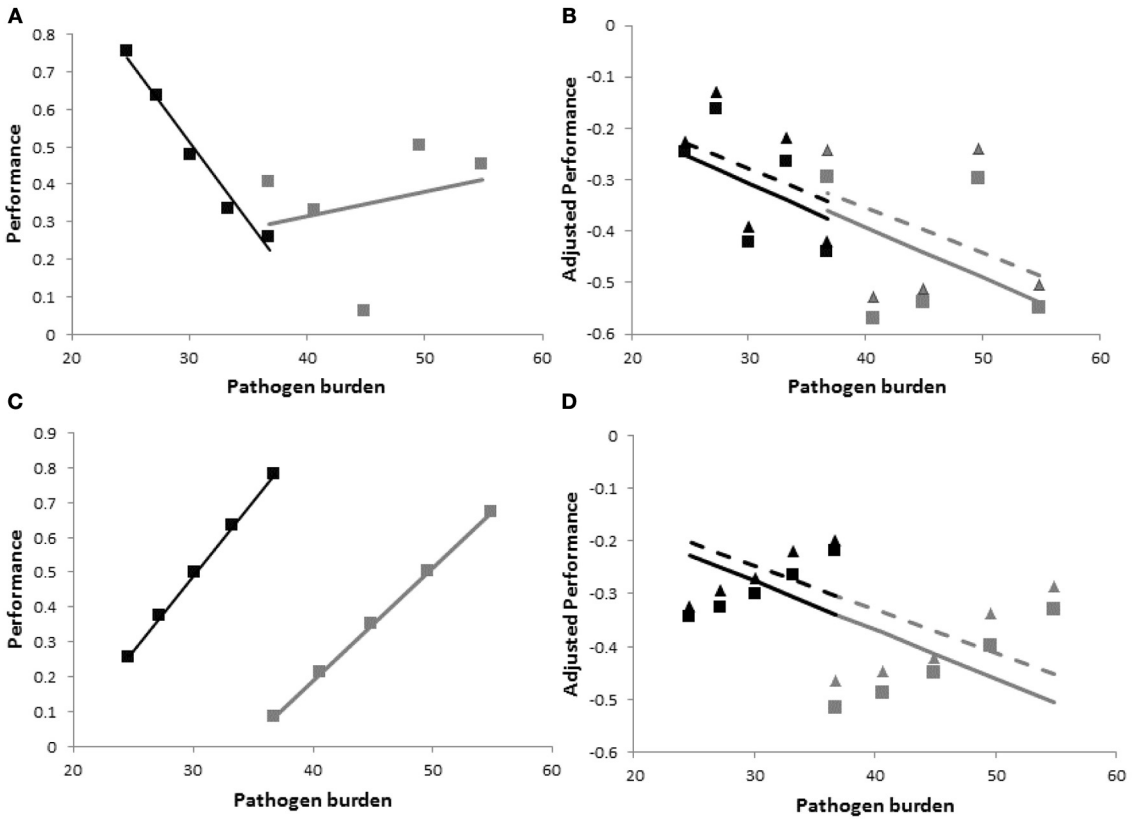

FIGURE 2 | Schematic figure to illustrate the importance of accounting for host variation in vigor, and the impact of changes in pathogen virulence and of correlation between resistance, vigor, and tolerance on resulting tolerance estimates. The panels show hypothetical performance vs. pathogen burden plots for estimating tolerance on a family level with individual variation in resistance, vigor and tolerance. Black and gray symbols refer to individual performance vs. pathogen burden measurements at an arbitrary time point from members of two families consisting of five individuals each. Black and gray lines are the corresponding regression lines whose slope values provide the family specific tolerance estimates. The families differ in average resistance, but have the same average vigor and tolerance, and the same trait within family variances and co-variances. Top (**A** and **B**) and bottom (**C** and **D**) panels refer to zero and strong correlations between resistance, vigor and tolerance, respectively. Panels on the left (**A** and **C**) show actual (non-adjusted) performance vs. pathogen burden and panels on the right (**B** and **D**) show performance adjusted to account for individual variation in vigor $y_{ij,adj} = y_{ij} - y0_{ij}$. Only the right hand panels (i.e., using adjusted performance) provide accurate tolerance estimates. The triangles and stippled lines in panels **(B)** and **(D)** include host-induced changes in pathogen virulence in the expressions for tolerance (i.e., $v_{ij} > 0$ in Equation 2). These increase within family variation in performance, but do not affect resulting tolerance estimates. Information on how the data were generated can be found in the Appendix.

over time. Thus, negative b_{ij} corresponds to incomplete tolerance and negative $v_{ij}(t)$ corresponds to a reduction in the impact of pathogen burden on performance over time. If $v_{ij}(t) = 0$, i.e., pathogen virulence does not change throughout the time-course of infection, Equation (2) reduces to (1). As illustrated by the triangle symbols in **Figures 2B,D** for $v_{ij}(t) = v_{ij} \times t$ with constant rates v_{ij}, changes in pathogen virulence alters host performance measured at a specific point in time without affecting the corresponding pathogen burden, and thus affects the resulting regression slopes derived from the scatter plots. This justifies statistically the inclusion of host-induced change in pathogen virulence as a component of tolerance.

It is noteworthy that the additional tolerance component introduces a further source of within family variation (and co-variation with other parameters), increasing thus the risk of errors in estimating tolerance slopes and the need for more samples to achieve statistical significance. In our simple illustrative example consisting of families with five individuals, the corresponding regression slopes were statistically significantly different ($p < 0.05$) when within family variation in pathogen virulence was added, although the average tolerance [i.e., average values for parameters b and v in equation (2)] was the same for both families. Note also that it is not possible to separate the two tolerance components (one affecting performance as a result of changing pathogen burden and one affecting the impact of a unit of pathogens on performance) when estimating group tolerance from the scatter plots. It is thus concluded that it is important to take into account that group tolerance estimates not only comprise changes in performance directly caused by changes in pathogen burden, but also by potential changes in the impact of the pathogens on host performance over time, and that both components may give rise to substantial within and between group variation.

THE INFLUENCE OF INDIVIDUAL VARIATION IN THE ONSET OF INFECTION ON THE TOLERANCE PHENOTYPE OF A GROUP

In all examples shown thus far it was assumed that all individuals become infected at the same time, and that measurements are taken at the same time post-infection. Whilst these conditions can be met in artificial challenge experiments (e.g., Råberg et al., 2007; Lefèvre et al., 2011), they are unlikely to hold in natural populations where the infection spreads naturally between individuals (e.g., Blanchet et al., 2010). Can reliable estimates of group tolerance be also obtained in the case of natural transmission dynamics based on samples collected at fixed time points? Two phenomena may interfere with the estimation: firstly, not all individuals of the population may have become infected, and thus not all individuals may express tolerance. Secondly, individuals are likely to vary in exposure and consequently become infected at different times, and the onset of infection is usually unknown. The first phenomenon is likely to affect the number of samples required to achieve statistical significance. To understand the impact of the second phenomenon on group tolerance, imagine for example, two individuals with the same resistance and tolerance. If infected at the same time, the individuals would produce the same point on the performance vs. pathogen burden plot. However, the individuals may have drastically different within host pathogen burden if they became infected at different times. Without knowing the onset of infection for both individuals, it is impossible to discern whether differences in within host pathogen burden reflect differences in host resistance or differences in exposure to infection. This may introduce complications for disentangling host resistance from tolerance and produce biased tolerance phenotypes (see section "Specifying Pathogen Burden"). Similarly, both individuals may have similar pathogen burden at the time of measurement, but due to different exposures, one individual is at the early stage of infection (e.g., when pathogen burden rises in **Figure 1A**), whereas the other individual is already in the process of recovery (e.g., when pathogen burden declines in **Figure 1A**). Furthermore, if the host immune response alters pathogen virulence over the time course of infection, the individual who is at the early infection stage is likely to have a greater performance measure than the recovering individual who has been infected for longer. In this case, differences in performance rather than in pathogen burden would produce artificial differences in the tolerance slopes.

To further illustrate the impact of different exposure times on group tolerance estimates, **Figure 3** shows cross-sectional samples of performance and pathogen burden for the same individuals of two families as simulated in **Figure 2B** (i.e., same average tolerance). However, in **Figure 3**, individuals were assumed to vary in their time of infection. This was represented by choosing the time of infection of each individual at random within a 5 day period. As a result, the corresponding family specific regression lines were no longer parallel, thus erroneously implying that one family is more tolerant than the other. In summary, individual variation in exposure blurs the distinction between resistance and tolerance and is likely to introduce bias in the phenotypic estimates of group tolerance.

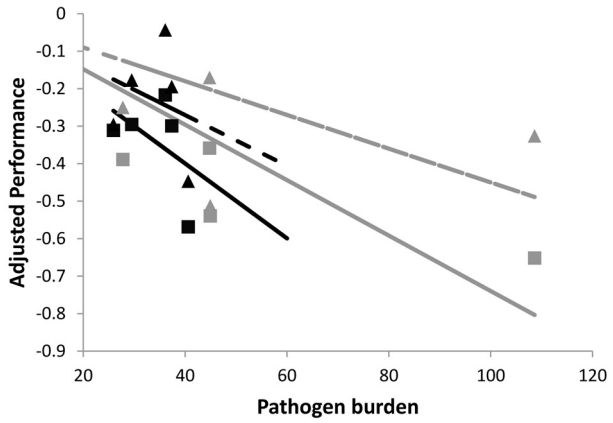

FIGURE 3 | Schematic figure to illustrate the importance of measuring pathogen burden and performance at the same time point post-infection. The panels shows simulated performance vs. pathogen burden plots for the same individuals of the two families whose plots are shown in **Figure 2B** (also solid lines corresponding to no change in pathogen virulence). Families differ in average resistance but have the same average tolerance. Here, pathogen burden and performance records refer to different time points post infection, mimicking natural field conditions where individuals get infected at different times. This introduces error in the family specific tolerance estimates, as reflected by the different slopes of the corresponding (black and gray) regression lines. For further explanation see caption of **Figure 2**.

DISCUSSION AND IMPLICATIONS

Reliable phenotypes are paramount for estimating genetic variation of traits of interest and for predicting breeding values for artificial selection. Improving tolerance of farm animals to infectious disease appears a desirable breeding goal for several reasons. However, apart from few studies investigating evidence for genetic variation in model species (e.g., Corby-Harris et al., 2007; Råberg et al., 2007; Ayres and Schneider, 2008) or wild populations (e.g., Blanchet et al., 2010; Lefèvre et al., 2011), essential knowledge of the existence and degree of genetic variation in tolerance of livestock is still lacking. We came to the conclusion that this gap of understanding may be largely due to difficulties in estimating tolerance phenotypes.

Most publications address the issue of "how to measure tolerance" either from a conceptual (e.g., Simms, 2000; Råberg et al., 2009; Ayres and Schneider, 2012) or an empirical perspective (e.g., Simms and Triplett, 1994; Mauricio et al., 1997; Råberg et al., 2007; Blanchet et al., 2010; Lefèvre et al., 2011). Conceptual studies are valuable for introducing the methodology (e.g., that tolerance can be considered as the slope when plotting performance vs. pathogen burden). However, they may not always reveal how these concepts can be implemented in practice. For example, tolerance as a concept is typically introduced at the level of individuals, but in practice it has only been estimated at the level of a group of individuals. Empirical studies, on the other hand may provide quantitative estimates of tolerance, but do not provide the necessary insight into the potential bias in these estimates as developed above. Tolerance estimates could be influenced by a variety of factors, including the time(s) at which measurements

are obtained, within family variation in resistance, tolerance and vigor, and co-variation in these traits as discussed above. It is difficult to determine the impact of these factors empirically. For this reason, we combined here a qualitative literature review with some simple simulations that allow a systematic and quantitative investigation of the effects of various individual factors and their interactions on resulting tolerance estimates.

Our study emphasizes that, in comparison to other traits targeted for genetic improvement in farm animals, estimating tolerance to infectious pathogens is more complicated as it requires multiple measurements per individual. This is even the case if tolerance is defined at a group level, and contrasts with, for example, estimates of resistance that can be obtained by measuring pathogen load at a relevant point in time. For instance, in order to avoid confounding effects between host resistance and tolerance, it is critical to measure not only the performance of individuals challenged with pathogens, but also their individual within host pathogen burden in a way that it accurately reflects host resistance. Also, in order to avoid bias in the tolerance slope estimates, it is essential to record individual host performance not only when individuals are infected, but also in a non-infected state or when exposed to a different level of pathogen challenge.

We are not the first to point out that measurements of within host pathogen burdens are critical for estimating group tolerance (e.g., Simms and Triplett, 1994; Råberg et al., 2009; Ayres and Schneider, 2012; Kause and Ødegård, 2012). Indeed, most empirical evidence for genetic variation in tolerance in plants and animals to date is based on analysis of covariance, where a significant F-test for family by pathogen burden interactions implies genetic variation in tolerance. There is however ambiguity in how and when within host pathogen burden should be measured. Previous studies have used (i) pathogen (e.g., macro parasite) levels at a particular time post-infection (e.g., Simms and Triplett, 1994; Mauricio et al., 1997; Pilson, 2000), (ii) peak pathogen burden (e.g., Råberg et al., 2007), (iii) the area of the pathogen curve over the time course of infection (e.g., Rowland et al., this issue), (iv) inoculation dose (e.g., Lefèvre et al., 2011), or (v) pathogen burden of individuals infected at different time points, measured at a fixed sampling time or after death (e.g., Blanchet et al., 2010; Lefèvre et al., 2011). Our study would suggest that options (i–iii) are the least likely to introduce bias in the resulting tolerance estimates, as they do not confound resistance and tolerance effects and do not simultaneously consider individuals that differ in their infection states. In particular, option (iii) would provide estimates of host resistance and tolerance that refer to the whole time period of infection rather than to a single point in time. However, this would require repeated measures of pathogen burden and host performance over time for every individual. Controlling the time at which records are collected may not always be feasible in practice; in particular if diagnostic tests for living animals do not exist for the infection under consideration. Also, available diagnostic tests may only provide crude estimates or proxies of actual pathogen burden and thus host resistance (e.g., PCR or ELISA test providing information of whether the animal has been infected or not). Further studies would be warranted to determine how inaccuracies in pathogen burden influence the resulting tolerance estimates.

Previous studies have demonstrated that ignoring individual variation in vigor can affect inferences about host evolution (Little et al., 2010), and introduce bias in estimates of genetic variance of tolerance when vigor and resistance are correlated (Kause, 2011). Our simulations show that serious bias in the tolerance slope estimates (and therefore probably also in the estimates of genetic variance in tolerance) can occur if individual variation in host vigor is not properly accounted for. As an individual cannot be simultaneously infected and not infected, these multiple measurements on an individual would need to be obtained prior (to measure vigor) and post (to measure tolerance) challenge. This may not be difficult to achieve, particularly if challenge tests are performed. However, as discussed in our companion paper (Doeschl-Wilson et al., 2012), the time delay between successive measures may introduce the risk that factors other than pathogen burden contribute to changes in host performance leading thus to biased tolerance slope estimates. This problem is easily prevented by choosing a performance trait that is zero for all animals in the absence of pathogen challenge, such as for example infection-induced weight loss (Råberg et al., 2007) or infection-induced mortality (e.g., Corby-Harris et al., 2007; Ayres and Schneider, 2008; Blanchet et al., 2010). Note however, that not all performance traits relevant in livestock production satisfy this criterion as they are rarely in a steady state. Hence, care needs to be taken in the statistical analysis to account for factors influencing temporal changes in performance not related to pathogen challenge.

For ease of illustration we assumed a linear relationship between pathogen burden and performance in our simulations. In reality, pathogen burden may have a non-linear effect on performance. In particular, it is quite likely that pathogen burden needs to exceed a certain threshold level within the host, before impacting noticeably on performance (Sandberg et al., 2006). Also, variation in pathogen virulence between hosts may cause a more complex relationship between host performance and pathogen burden in the scatter plots that cannot be easily linearized. Our conclusions should also hold in the case of such non-linear relationships, although adaptations in the quantification of tolerance would need to be made because the slope will no longer be constant over the entire pathogen burden range. Two approaches for dealing with such non-linear relationships are presented in the literature. The first approach restricts the definition of tolerance to a range of pathogen burden values over which the slope is approximately constant [termed "range tolerance" by Little et al. (2010)]. This would imply that in order to compare tolerance of different groups of individuals, the groups need to overlap in their levels of pathogen burden. Otherwise, they may be equally tolerant but their data may refer to different sections of the performance vs. pathogen burden curve and conclusions obtained will be wrong (see e.g., Råberg et al., 2009). The second approach is to replace the slope of the regression of performance against pathogen burden with the area of performance under the pathogen burden curve, after standardizing to account for variation in vigor (Pilson, 2000).

Accurate phenotypic measures only constitute the first step toward predicting breeding values for artificial selection or for identifying loci affecting the trait under consideration. The accuracy of these genetic parameter estimates will not only depend

on the quality of resistance and performance measures, but also on family size, genetic, and phenotypic correlations between this trait and resistance and vigor, and on the underlying genetic architecture (Kause, 2011). Perhaps the most limiting factor in genetic improvement of host tolerance is the fact that tolerance as a trait is a property of an individual, yet according to current methods it can only be quantified at a group level. In current breeding programs, selection across families is used as an alternative to individual selection when traits cannot be measured on selection candidates (e.g., meat quality and disease resistance traits). However, the genetic progress that can be achieved by artificial selection is limited when within family variation is ignored. Genome-wide evaluations are considered highly beneficial in such cases where individual phenotypes are difficult to obtain in practice, as they provide a means to use both between and within family variation (Sonesson and Meuwissen, 2009; Villanueva et al., 2011). But although the need of measuring the trait of interest can be avoided for some generations, individual measures are still needed for estimating SNP effects. Thus for genetic improvement programs, individual tolerance phenotypes would be highly desirable.

In conclusion, estimating tolerance phenotypes for a group of related individuals constitutes an important first step toward improving the tolerance of livestock to infectious diseases. In order to obtain unbiased estimates of group tolerance, accurate measures of within host pathogen burden and performance of individual animals, associated with different levels of pathogen burden (e.g., non-infected and infected) are needed. In order to avoid confounding effects between differences in individual resistance and environmental exposure when estimating group tolerance, individual measures of pathogen burden and host performance would need to be obtained at the same time point post pathogen exposure for all individuals. This makes estimating tolerance from field data extremely challenging.

It should be noted, that many of the issues raised here for tolerance also arise when improving host genetic resistance to infectious disease through selection (Bishop et al., 2012). In particular, as outlined by Bishop and Woolliams (2010) individual differences in pathogen exposure, appropriate timing of measurement and poor test diagnostics all contribute to potential bias in genetic parameter estimates for host resistance to infectious pathogens. Nevertheless, selection for improved host resistance has been notably successful for a variety of diseases, including nematode infections in sheep, IPN in salmon, mastitis in dairy cattle and *E. coli* infections in pigs. Although natural selection for tolerance appears to have been successful in several animal and plant species, it remains to be shown if similar success can be achieved through artificial selection. This paper contributes toward this endeavor by outlining the kind of measurements needed to make progress in this direction.

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REFERENCES

Agrawal, A. A., Strauss, S. Y., and Stout, M. J. (1999). Costs of induced responses and tolerance to herbivory in male and female fitness components of wild radish. *Evolution* 53, 1093–1104.

Albers, G. A. A., and Gray, G. D. (1986). "Breeding for worm resistance: a perspective," in *Parasitoly – Quo Vadit?* ed M. J. Howell (Canberra: Australian Academy of Science), 559–566.

Ayres, J. S., and Schneider, D. S. (2008). A signaling protease required for melanization in Drosophila affects resistance and tolerance of infections. *PLoS Biol.* 6:e305. doi: 10.1371/journal.pbio.0060305

Ayres, J. S., and Schneider, D. S. (2012). Tolerance of infections. *Annu. Rev. Immunol.* 30, 271–294.

Baylis, M., Chihota, C., Stevenson, E., Goldman, W., Smith, A., Sivam, K., et al. (2004). Risk of scrapie in British sheep of different prion protein genotype. *J. Gen. Virol.* 85, 2735–2740.

Best, A., White, A., and Boots, M. (2008). Maintenance of host variation in tolerance to pathogens and parasites. *Proc. Natl. Acad. Sci. U.S.A.* 105, 786–791.

Bishop, S. C. (2012). A consideration of resistance and tolerance for ruminant nematode infections. *Front. Gene.* 3:168. doi: 10.3389/fgene.2012.00168

Bishop, S. C., Doeschl-Wilson, A. B., and Woolliams, J. A. (2012). Uses and implications of field disease data for livestock genomic and genetics studies. *Front. Gene.* 3:114. doi: 10.3389/fgene.2012.00114.

Bishop, S. C., and Woolliams, J. A. (2010). On the genetic interpretation of disease data. *PLoS ONE* 5:e8940. doi: 10.1371/journal.pone.0008940

Bisset, S. A., and Morris, C. A. (1996). Feasibility and implications of breeding sheep for resilience to nematode challenge. *Int. J. Parasitol.* 26, 857–868.

Blanchet, S., Rey, O., and Loot, G. (2010). Evidence for host variation in parasite tolerance in a wild fish population. *Evol. Ecol.* 24, 1129–1139.

Caldwell, R., Compton, L., and Patterson, F. (1958). Tolerance to cereal leaf rusts. *Science* 128, 714–715.

Corby-Harris, V., Habel, K., Ali, F., and Promislow, D. (2007). Alternative measures of response to *Pseudomonas aeruginosa* infection in *Drosophila melanogaster*. *J. Evol. Biol.* 20, 526–533.

Detilleux, J. (2011). Effectiveness analysis of resistance and tolerance to infection. *Genet. Sel. Evol.* 43, 9.

Doeschl-Wilson, A. B., Bishop, S. C., Kyriazakis, I., and Villanueva, B. (2012). Novel methods for quantifying individual host response to infectious pathogens for genetic analyses. *Front. Gene.* 3:266. doi: 10.3389/fgene.2012.00266

Doeschl-Wilson, A. B., Vagenas, D., Kyriazakis, I., and Bishop, S. C. (2008). Challenging the assumptions underlying genetic variation in host nematode resistance. *Genet. Sel. Evol.* 40, 241–264.

Doeschl-Wilson, A. B., Brindle, W., Emmans, G., and Kyriazakis, I. (2009a). Unravelling the relationship between animal growth and immune response during micro-parasitic infections. *PLoS ONE* 4:e7508. doi: 10.1371/journal.pone.0007508

Doeschl-Wilson, A. B., Kyriazakis, I., Vincent, A., Rothschild, M. F., Thacker, E., and Galina-Pantoja, L. (2009b). Clinical and pathological responses of pigs from two genetically diverse commercial lines to porcine reproductive and respiratory syndrome virus infection. *J. Anim. Sci.* 87, 1638–1647.

Fineblum, W. L., and Rausher, M. D. (1995). Trade-off between resistance and tolerance to herbivore damage in a morning glory. *Nature* 377, 517–520.

Foresight annual review. (2011). *The Foresight Programme*. UK Government Office for Science. Available online at: http://www.bis.gov.uk/foresight/publications/annual-reviews/annual-review-2011

Houston, R. D., Haley, C. S., Hamilton, A., Guy, D. R., Mota-Velasco, J., Gheyas, A., et al. (2010). The susceptibility of Atlantic salmon fry to freshwater Infectious Pancreatic Necrosis is largely explained by a major QTL. *Heredity* 105, 318–327.

Kause, A. (2011). Genetic analysis of tolerance to infections using random regressions: a simulation study. *Genet. Res.* 93, 291–302.

Kause, A., and Ødegård, J. (2012). The genetic analysis of tolerance to

infections: a review. *Front. Gene.* 3:262. doi: 10.3389/fgene.2012.00262

Keeling, M. J., and Rohani, P. (2008). *Modelling Infectious Diseases in Humans and Animals*. Princeton: Princeton University Press.

Kemp, S. J., and Teale, A. J. (1998). Genetic basis of Trypanotolerance in cattle and mice. *Parasitol. Today* 14, 450–454.

Koskela, T., Puustinen, S., Salonen, V., and Mutikainen, P. (2002). Resistance and tolerance in a host plant–holoparasitic plant interaction: genetic variation and costs. *Evolution* 56, 899–908.

Kover, P. X., and Schaal, B. A. (2002). Genetic variation for disease resistance and tolerance among *Arabidopsis thaliana* accessions. *Proc. Natl. Acad. Sci. U.S.A.* 99, 270–274.

Lefèvre, T., Williams, A. J., and de Roode, J. C. (2011). Genetic variation in resistance, but not tolerance, to a protozoan parasite in the monarch butterfly. *Proc. R. Soc. B* 278, 751–759.

Lewis, C. R. G., Torremorell, M., Galina-Pantoja, L., and Bishop, S. C. (2009). Genetic parameters for performance traits in commercial sows estimated before and after an outbreak of porcine reproductive and respiratory syndrome. *J. Anim. Sci.* 87, 876–884.

Little, T. J., Shuker, D. M., Colegrave, N., Day, T., and Graham, A. L. (2010). The coevolution of virulence: tolerance in perspective. *PLoS Pathog*. 6:e1001006. doi: 10.1371/journal.ppat.1001006

MacKenzie, K., and Bishop, S. C. (1999). A discrete-time epidemiological model to quantify selection for disease resistance. *Anim. Sci.* 69, 543–551.

Mackinnon, M. J., Meyer, K., and Hetzel, D. J. S. (1991). Genetic variation and covariation for growth, parasite resistance and heat tolerance in tropical cattle. *Livest. Prod. Sci.* 27, 105–122.

Mauricio, R., Rausher, M. D., and Burdick, D. S. (1997). Variation in the defense strategies of plants: are resistance and tolerance mutually exclusive? *Ecology* 78, 1301–1311.

McIntyre, J. D., and Amend, D. F. (1978). Heritability of tolerance for infectious hematopoietic necrosis in Sockeye salman (*Oncorhynchus nerka*). *Am. Fish. Soc.* 107, 305–308.

Medszhitov, R., Schneider, D. S., and Soares, M. P. (2012). Disease tolerance as a defense strategy. *Science* 335, 936.

Meijerink, E., Fries, R., Vogeli, P., Masabanda, J., Wiager, G., Stricker, C., et al. (1997). Two α (1, 2) fucosyltransferase genes on porcinechromosome 6q11 are closely linked to the blood group inhibitor(S) and *Escherichia coli* F18 receptor (ECF18R) Loci. *Mamm. Genome* 8, 736–741.

Morris, C. A. (2007). A review of genetic resistance to disease in Bos taurus cattle. *Vet. J.* 174, 481–491.

Naessens, J. (2006). Bovine trypanotolerance: a natural ability to prevent severe anaemia and haemophagocytic syndrome? *Int. J. Parasitol.* 36, 521–528.

Ødegård, J., Baranski, M., Gjerde, B., and Gjedrem, T. (2011). Methodology for genetic evaluation of disease resistance in aquaculture species: challenges and future prospects. *Aquacult. Res.* 42, 103–114.

Ødegård, J., Gitterle, T., Madsen, P., Meuwissen, T. H. E., Yazdi, H. Y., Gjerde, B., et al. (2011). Quantitative genetics of taura syndrome resistance in pacificwhite shrimp (*Penaeusvannamei*): a cure model approach. *Genet. Sel. Evol.* 43, 14.

Ødegård, J., Yazdi, M. H., Sonesson, A. K., and Meuwissen, T. H. E. (2009). Incorporating desirable genetic characteristics from an inferior into a superior population using genomic selection. *Genetics* 181, 737–745.

Pilson, D. (2000). The evolution of plant responses to herbivory: simultaneously considering resistance and tolerance in *Brassica rapa*. *Evol. Ecol.*14, 457–489.

Råberg, L., Graham, A. L., and Read, A. F. (2009). Decomposing health: tolerance and resistance to parasites in animals. *Philos. Trans. R. Soc. B* 364, 37–49.

Råberg, L., Sim, D., and Read, A. F. (2007). Disentangling genetic variation for resistance and tolerance to infectious disease in animals. *Science* 318, 318–320.

Rausher, M. D. (2001). Co-evolution and plant resistance to natural enemies. *Nature* 411, 857–864.

Read, A. F., Graham, A. L., and Råberg, L. (2008). Animal defenses against infectious agents: is damage control more important than pathogen control? *PLoS Biol*. 6:e4. doi: 10.1371/journal.pbio.1000004

Roy, B. A., and Kirchner, J. W. (2000). Evolutionary dynamics of pathogen resistance and tolerance. *Evolution* 54, 51–63.

Sandberg, F. B., Emmans, G. C., and Kyriazakis, I. (2006). A model for predicting food intake of growing animals during exposure to pathogens. *J. Anim. Sci.* 84, 1552–1566.

Schafer, J. (1971). Tolerance to plant disease. *Annu. Rev. Phytopathol.* 9, 235–252.

Schneider, D. (2011). Tracing personalized health curves during infections. *PLoS Biol.* 9:e1001158. doi: 10.1371/journal.pbio.1001158

Simms, E. L. (2000). Defining tolerance as a norm of reaction. *Evol. Ecol.* 14, 563–570.

Simms, E. L., and Triplett, J. (1994). Costs and benefits of plant responses to disease-resistance and tolerance. *Evolution* 48, 1973–1985.

Sonesson, A. K., and Meuwissen, T. H. E. (2009). Testing strategies for genomic selection in aquaculture breeding programs. *Genet. Sel. Evol.* 41, 37.

Stowe, K., Marquis, R., Hochwender, C., and Simms, E. L. (2000). The evolutionary ecology of tolerance to consumer demand. *Annu. Rev. Ecol. Syst.* 3, 565.

Tiffin, P. (2000). Mechanisms of tolerance to herbivore damage: what do we know? *Evol. Ecol.* 14, 523–536.

Tiffin, P., and Rausher, M. D. (1999). Genetic constraints and selection acting on tolerance to herbivory in the common morning glory Ipomoea purpurea. *Am. Nat.* 154, 700–716.

Vagenas, D., Bishop, S. C., and Kyriazakis. I. (2007). A model to account for the consequences of host nutrition on the outcome of gastrointestinal parasitism in sheep: model evaluation. *Parasitology* 134, 1279–1289.

Van der Waaij, E. H., Bijma, P., Bishop, S. C., and van Arendonk, J. A. M. (2000). Modeling selection for production traits under constant infection pressure. *J. Anim. Sci.* 78, 2809–2820.

Via, S., and Lande, R. (1985). Genotype-environment interaction and the evolution of phenotypic plasticity. *Evolution* 39, 505–522.

Villanueva, B., Fernández, J., García-Cortés, L. A., Varona, L., Daetwyler, H. D., and Toro, M. A. (2011). Accuracy of genome-wide evaluation for disease resistance in aquaculture breeding programmes. *J. Anim. Sci.* 89, 3433–3442.

Zelnik, V. (2003). Marek's disease research in the post-sequencing era: new tools for the study of gene function and virus-host interactions. *Avian Pathol.* 32, 323–333.

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APPENDIX

SUPPLEMENTARY INFORMATION ON GENERATION OF DATA FOR FIGURES 1 AND 2, RESPECTIVELY

The data for **Figures 1** and **2** were generated using the following equations for pathogen burden (PB_{ij}) of individual i in family j and performance (y_{ij}):

$$PB_{ij}(t) = 100t^2 exp\left(-c_{ij}t\right)$$
$$y_{ij}(t) = y0_{ij}(t) - k_{ij}(t)PB_{ij}(t)$$

where $k_{ij}(t) = b_{ij} - v_{ij}t$ refers to the tolerance slope, and $y0_{ij}(t)$ refers to performance at time t in the absence of pathogen challenge.

Parameter values for individuals 1 and 2 in **Figure 1** were $c_1 = 0.8$ and $c_2 = 0.8$, $v_i = 0$ for $i = 1, 2$ and $y0_1 = 1$ and $y0_2 = 0.5$, respectively.

Parameter values for individuals in **Figure 2** were

$$c_{i1} = \{0.6, 0.7, 0.8, 0.9, 1.0\}, \; c_{i2} = \{1.0, 1.1, 1.2, 1.3, 1.4\},$$
$$y0_{ij} \in \{0.6, 0.7, 0.8, 0.9, 1.0\}, \; b_{ij} = \{0.006, 0.008, 0.01, 0.012, 0.014\},$$

and $v_{ij} = 0$ (squared symbols and solid lines) or v_{ij} ε {0.0006, 0.0008, 0.001, 0.0012, 0.0014} for triangles and stippled lines. For **Figures 1A,B**, individual parameter values were chosen at random, whereas for **Figures 1C,D**, individual parameter values were ordered to generate correlation between resistance, tolerance and vigor. Plots were generated based on predicted pathogen burden and performance at time $t = 1$.

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Novel methods for quantifying individual host response to infectious pathogens for genetic analyses

Andrea B. Doeschl-Wilson[1]*, Steve C. Bishop[1], Ilias Kyriazakis[2] and Beatriz Villanueva[3]

[1] The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, UK
[2] School of Agriculture, Food and Rural Development, Newcastle University, Newcastle upon Tyne, UK
[3] Departamento de Mejora Genética Animal, INIA, Madrid, Spain

Edited by:
Guilherme J. M. Rosa, University of Wisconsin-Madison, USA

Reviewed by:
Selma Forni, Genus Plc, USA
Georgios Banos, Aristotle University of Thessaloniki, Greece

***Correspondence:**
Andrea B. Doeschl-Wilson, The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush, Midlothian, EH26 9RG, UK.
e-mail: andrea.wilson@roslin.ed.ac.uk

We propose two novel approaches for describing and quantifying the response of individual hosts to pathogen challenge in terms of infection severity and impact on host performance. The first approach is a direct extension of the methodology for estimating group tolerance (the change in performance with respect to changes in pathogen burden in a host population) to the level of individuals. The second approach aims to capture the dynamic aspects of individual resistance and tolerance over the entire time course of infections. In contrast to the first approach, which provides a means to disentangle host resistance from tolerance, the second approach focuses on the combined effects of both characteristics. Both approaches provide new individual phenotypes for subsequent genetic analyses and come with specific data requirements. In particular, both approaches rely on the availability of repeated performance and pathogen burden measurements of individuals over the time course of one or several episodes of infection. Consideration of individual tolerance also highlights some of the assumptions hidden within the concept of group tolerance, indicating where care needs to be taken in trait definition and measurement.

Keywords: infectious disease, host–pathogen interaction, tolerance, resistance, random regression, dynamical system, infection dynamics, breeding for disease resistance

INTRODUCTION

Resistance and tolerance to infectious pathogens are important characteristics of livestock to counteract the potential detrimental impact of pathogens on animal health and production. Host resistance refers to the ability to reduce pathogen replication, in the broadest sense, whereas tolerance refers to the ability to reduce the impact of pathogens on host performance without necessarily affecting pathogen burden. In order to target these characteristics for genetic improvement, they need to be quantifiable. Host resistance is generally quantified by a measure of infection severity such as within-host pathogen burden (e.g., viral or bacterial counts or parasite density). Tolerance may be quantitatively defined as the change in host performance with respect to change in pathogen burden (e.g., Simms, 2000), where performance may refer to any trait relevant for production or reproduction (e.g., growth rate, feed intake, or litter size).

As outlined in our companion paper (Doeschl-Wilson et al., 2012), although conceptually tolerance is defined at the individual level (Simms, 2000; Schneider and Ayres, 2008), empirical tolerance estimates have only been obtained at the level of groups of (related) individuals. In particular, group tolerance estimates are usually derived using analysis of covariance (ANCOVA) or random regression approaches, where performance measures of infected group members are regressed against their respective pathogen burden at a given time post infection (e.g., Simms and Triplett, 1994; Mauricio et al., 1997; Råberg et al., 2007; Kause, 2011). Whilst these approaches may provide useful evidence on whether genetic variation in tolerance exists, the resulting group estimates have several disadvantages that render them not ideal for genetic studies, for the three reasons outlined below.

The first issue is that these estimates rely on the underlying assumption that all animals have been exposed to, or infected with, the same dose/type of pathogens at the same time. This assumption is unlikely to hold in field conditions and it raises the question of whether reliable group tolerance estimates can be obtained from field data (Doeschl-Wilson et al., 2012), which are the primary data source for quantitative genetic analyses of disease traits (Bishop and Woolliams, 2010).

Secondly, group tolerance estimates, which are usually obtained from cross-sectional measures (i.e., measures taken at one time point during the infection) (Simms and Triplett, 1994; Mauricio et al., 1997; Råberg et al., 2007; Ayres and Schneider, 2012), may poorly represent the overall impact of infection on host performance over the entire time course of infection. Consider for example two families with equal average resistance, i.e., the same pathogen burden profiles. Assume however that members of family A have a significantly greater ability to prevent tissue damage inflicted by pathogens than members of family B but members of family B have developed more efficient recovery mechanisms (e.g., tissue repair mechanisms) than those belonging to family A. Thus, ANCOVA or random regression may indicate significant family differences in tolerance depending on when during the infection process the performance and pathogen

burden records were taken. Based on early measurements family A would be selected as more tolerant, whereas based on late measurements members of family B may have emerged as favorable selection candidates. The example illustrates that the effects of host tolerance may vary throughout the infection as a result of different mechanisms acting at different stages. Measurements taken at different stages of the infection may therefore give rise to different tolerance estimates and hence also to different estimates of genetic variance in tolerance. Biased estimates of genetic parameters may therefore arise if these dynamic changes are neglected. In principle, group tolerance estimates that describe the impact of infection on performance over the entire time course of one or several episodes of infection could be obtained by using cumulative measures of performance and pathogen burden over time rather than cross-sectional measures, as suggested by Ayres and Schneider (2012). However, cumulative measures would require repeated measurements of both host performance and pathogen burden on infected individuals, in addition to measurements of host performance in the absence of pathogen challenge (or in less pathogenic environments), as established in our companion paper (Doeschl-Wilson et al., 2012). In summary, in order to obtain a reliable estimate of tolerance at the *group* level a multitude of measurements at the *individual* level would be needed. This seems a disproportionately large effort for the limited genetic gain that can be achieved with group selection.

Finally, from an animal breeding perspective, another major drawback of group rather than individual tolerance is that selection accuracy and therefore response to selection is limited if phenotypes are specified at the group level (Falconer and Mackay, 1996). Furthermore, group phenotypes do not take advantage of the benefits of recent advances in high throughput genomics for dissecting host responses to infectious pathogens and increasing the accuracy of selection. For instance, increased availability of dense Single Nucleotide Polymorphism (SNP) chips in most livestock species has facilitated genome-wide prediction for obtaining accurate breeding values. For example, SNP markers whose effects have been calibrated in individuals that are both genotyped and measured for the trait of interest may then be used to obtain estimated breeding values for animals that are genotyped but not phenotyped. Thus, with genome-wide evaluations, obtaining phenotypes for disease traits (a difficult task in most cases) can be avoided for at least some generations before marker effects need to be re-estimated. Having only group tolerance phenotypes available implies that information arising from within-group variation cannot be exploited in the first step, thus sacrificing potential accuracy of selection.

In summary, the availability of individual phenotypes of both host resistance and tolerance would be highly desirable for quantifying infection severity and impact on production over the time course of one or several episodes of infection. Both resistance and tolerance are defined and expressed at the level of individuals. It is the limitation of current statistical approaches that restrict the estimation of host tolerance to the level of groups. In this article two novel approaches for estimating individual tolerance are proposed. Both approaches rely on the availability of repeated measurements of host performance and pathogen burden. The first approach is a direct extension of the methodology for estimating group tolerance to the level of individuals, and provides a means to disentangle host resistance from tolerance. The second approach aims to capture the combined dynamic effects of individual resistance and tolerance over the entire time course of infections in terms of infection severity and impact on performance.

EXTENDING THE STATISTICAL FRAMEWORK FROM GROUP TOLERANCE TO INDIVIDUAL TOLERANCE

The well-established definition of tolerance as the change in performance with respect to changes in pathogen burden (Simms, 2000) implies that to quantify tolerance at the level of individuals, repeated measurements of host performance and pathogen burden from individual animals would be needed. In other words, individual tolerance can only be quantified if within host pathogen burden changes over time, and both performance and pathogen burden can be measured repeatedly on each individual. Thus, traits that can only be measured once (e.g., carcass and survival traits) are not suitable for estimating individual tolerance. For repeatedly measurable performance traits (e.g., growth rate, milk yield, litter size), individual tolerance may describe how an individual's performance is affected over the time course of one infection, or over several episodes of infections, depending on when measurements are taken.

MATHEMATICAL FRAMEWORK FOR INDIVIDUAL TOLERANCE

Group tolerance is typically inferred by regressing measurements of host performance (usually collected at a specific time point post infection) against corresponding measurements of pathogen burden for individual group members (e.g., Simms, 2000; Råberg et al., 2007; Kause, 2011). The framework can be extended to estimate tolerance of individuals by replacing cross-sectional measurements of multiple individuals by multiple repeated measurements per individual. However, as a consequence of using repeated measurements taken at different time points, time would need to be included explicitly when describing the relationship between performance and pathogen burden. In other words, instead of fitting the linear model $y = y0 + bPB$ currently used to estimate group tolerance, which corresponds to a snapshot in time, a time-dependent model would need to be used:

$$y(t) = y0(t) - b(t)PB(t) \tag{1}$$

where $y0(t)$ denotes host performance at time t in a pathogen-free environment and $b(t)$ describes the effect of pathogen burden on host performance at time t. Several implications of using the time-dependent model (1) for estimating individual tolerance should be pointed out. Firstly, taking the first order derivative of $y(t)$ with respect to time, i.e.,

$$\frac{dy}{dt} = \frac{dy0}{dt} - \frac{db}{dt}PB - b\frac{dPB}{dt} \tag{2}$$

illustrates that a change in performance of an infected individual can be the result of three different causes:

1. A change in host performance over time not related to pathogen challenge ($\frac{dy0}{dt}$), i.e., a change that would also occur if the individual was not infected.
2. A change in the impact of a unit pathogen dose on host performance ($\frac{db}{dt}PB$), i.e., host-induced change in pathogen virulence over the time course of infection.
3. A change in host performance associated with changes in within-host pathogen burden ($b\frac{dPB}{dt}$).

Only the last two components, i.e., those including expressions of $b(t)$, are associated with tolerance mechanisms.

Secondly, Equation (2) reveals that in order to obtain accurate estimates of tolerance effects, natural temporal variations in host performance would need to be accounted for. It also reveals that tolerance, which is mathematically defined as $\frac{dy}{dPB}$ may change over time. This becomes evident from Equation (2) after expressing $\frac{dy}{dPB}$ as

$$\frac{dy}{dPB} = \frac{\frac{dy}{dt}}{\frac{dPB}{dt}} \tag{3}$$

Substituting Equation (2) into (3) shows that the mathematical definition of tolerance as the incremental change in performance with respect to pathogen burden, i.e., $\frac{dy}{dPB}$ is generally not the same as $b(t)$. Indeed, $\frac{dy}{dPB}$ is only equal to $b(t)$ if both $y0(t)$ (performance in the absence of pathogen challenge) and $b(t)$ (impact of pathogen burden on host performance) are constant over time (so that their derivatives with respect to time are 0). However, since $\frac{dy}{dPB}$ encompasses also changes in host performance not related to pathogen burden, tolerance may be more appropriately represented by the term $b(t)$. This is also consistent with the classical definition of tolerance as the slope b in Equation (1).

STATISTICAL FRAMEWORK FOR ESTIMATING INDIVIDUAL TOLERANCE

Random regression models have proved to be a useful framework for estimating tolerance of families of individuals (Kause, 2011; Kause and Ødegård, 2012). In particular, Kause (2011) demonstrated that the tolerance bj of a group j can be estimated as the regression slope of the linear model

$$y_{ij} = y0_{ij} - b_j PB_{ij} + e_{ij} \tag{4}$$

where y_{ij} and PB_{ij} refer to host performance and pathogen burden of individual i in group j obtained at a fixed time post infection, respectively. It has been also established that in order to obtain unbiased group tolerance estimates bj, estimates of host performance $y0_{ij}$ in a pathogen-free environment would be required (Kause, 2011; Doeschl-Wilson et al., 2012). Such estimates may be obtained simply from having group members in environments differing in pathogen challenge.

The statistical random regression framework can be extended to estimate tolerance of individuals by replacing the cross-sectional measurements y_{ij} and PB_{ij} of multiple individuals in Equation (4) by multiple repeated measurements per individual. Thus, the time-dependent mathematical model (1) relating individual host performance at time t to the corresponding pathogen burden can be represented as a statistical repeated measurement model as follows:

$$y_{ik} = y0_{ik} - b_{ik} PB_{ik} + e_{ik} \tag{5}$$

where y_{ik} and PB_{ik} are the performance and pathogen burden of individual i measured at discrete time t_k, respectively, $y0_{ik}$ is the performance of individual i at t_k in a pathogen-free environment (assumed to be known), b_{ik} represents the impact of pathogen burden at time t_k on host performance (to be estimated), and e_{ik} is the individual error at t_k (to be estimated).

In order to obtain estimates for the tolerance parameter b_{ik} further information or assumptions are required. Firstly, $y0_{ik}$ is assumed to be known. However, as a host cannot be simultaneously infected and non-infected, measures of $y0$ throughout the time period of infection do not exist and would thus need to be inferred from available measurements taken at different times. Thus, accurate tolerance estimates at the level of individuals can only be obtained for performance traits and time periods over which host performance in the absence of pathogens is known [e.g., weight loss in mature animals as used by Råberg et al. (2007)] or can be inferred based on measurements prior to infections (e.g., projected growth trajectory or milk yield).

Secondly, b_{ik} needs to be either assumed as constant over time (i.e., $b_{ik} = b_i$ for all t_k) or expressed in a form that can be included in the above model. Note that temporal variation in b corresponds to temporal variation in the impact that a unit dose of pathogens inflicts on host performance, i.e., temporal variation in host-induced pathogen virulence. Thus, a constant value b_i is justified in cases where the impact of a given pathogen burden does not change over time. This may be the case for example if the time interval over which tolerance is to be estimated is relatively short or if tolerance estimates refer to similar time points post infection during successive episodes of infection for individuals with no lasting immunity. The latter may apply to nematode infections in peri-parturient ewes or to mastitis in lactating cows. However, the assumption is less likely to hold if tolerance is to be estimated over the entire time course of one infection, as changes in the host immune response over time (e.g., mechanisms related to damage prevention or repair) would generally correspond to host-induced changes in pathogen virulence. However, in this case, i.e., when changes in pathogen virulence cannot be ignored, a simple expression for b_{ik} may be used. For example, adopting the principle of Occam's razor, b_{ik} in Equation (3) may be represented by

$$b_{ik} = b_i(t_k) = b_{0i} - v_i t_k \tag{6}$$

where b_{0i} describes the change in performance of individual i due to change in pathogen burden and $v_i t_k$ with constant rate parameter v_i describes the change in the impact of a unit dose of pathogens on performance. For parameter estimation, it would be more convenient if data were obtained from challenge experiments, where t_k refers to time post infection. Substitution of Equation (6) into (5), and appropriate specifications of variance and covariance structures for the individual parameters, subsequent measurements, and residuals, in principle yields individual estimates for the tolerance parameters b_{0i} and v_i, which

refer to different tolerance components. These estimates would constitute the phenotypes for subsequent genetic analyses. Exact data requirements for such an approach and properties of the obtained estimates have yet to be explored.

A NEW FRAMEWORK FOR QUANTIFYING INDIVIDUAL RESISTANCE AND TOLERANCE USING DYNAMICAL SYSTEMS THEORY

The statistical framework described above for quantifying individual (and group) tolerance relies on a number of stringent assumptions and measurement requirements. In particular, regression models assume a specific (direct) relationship between host performance and pathogen burden that can be formulated by a simple (often linear) mathematical function. This function may be a poor representation of the underlying biological processes affecting infection severity and impact on performance. For example, reduction in host performance may be caused by immune processes (Coop and Kyriazakis, 1999; Ayres and Schneider, 2012; Glass, 2012) rather than directly by pathogens. Although this was captured to some extent by Equation (6), one may question whether a statistical model that assumes a direct (and often linear) relationship between pathogen burden and performance can yield reliable tolerance estimates.

Furthermore, both resistance and tolerance usually relate to immunological processes (e.g., blocking pathogen entry in host target cell or reducing pathogen reproduction rate and preventing or repairing tissue damage) which are highly dynamic and often temporary. A conventional statistical framework may not be the most appropriate means of capturing these dynamic aspects. Instead, an alternative mathematical approach tailored toward dynamic processes may be better suited to capture the information revealed by repeated measurements of host pathogen burden and performance over time. This is outlined below.

PERFORMANCE vs. PATHOGEN BURDEN TRAJECTORIES

Scatter plots of individual performance *vs.* pathogen burden are fundamental for quantifying group tolerance (e.g., Simms, 2000; Råberg et al., 2007, 2009; Doeschl-Wilson et al., 2012). Adapting these plots to the level of an individual, i.e., by plotting the individual's performance *vs.* pathogen burden measurements collected at different time points, generates a trajectory in the pathogen burden–performance space (**Figures 1**, **2**). This trajectory can reveal important information on how the individual's resistance and tolerance mechanisms interact over time. Consider for example the growth rate–pathogen burden trajectories of the two pigs depicted in **Figure 1**. These trajectories were generated based on repeated measurements of body weight and virus load collected over a period of 42 days after challenging the pigs with a given dose of the Porcine Reproductive and Respiratory Syndrome virus [experiment described by Rowland et al. (2012)]. The trajectory of pig 1 shows that the pig apparently manages to clear the virus within the observation period of 42 days, but suffers a long-term reduction in growth (growth rate at the end of infection is negative). In contrast, the trajectory of pig 2 indicates that the pig did not manage to clear the virus within the 42 day observation period—in fact it seemingly

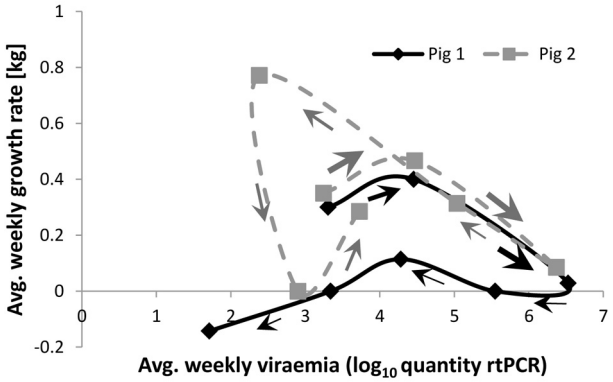

FIGURE 1 | Performance (average weekly growth rate) *vs.* pathogen burden (average weekly viraemia) trajectories for two individual pigs infected with the same challenge dose of a virulent PRRS virus strain. Arrows indicate the direction of growth rate-viraemia plots over time. The size of the arrows crudely reflect the speed at which the trajectory progresses. Data are courtesy of the PRRS Host Genetics Consortium—for more information about data acquisition and experimental design see Rowland et al. (2012).

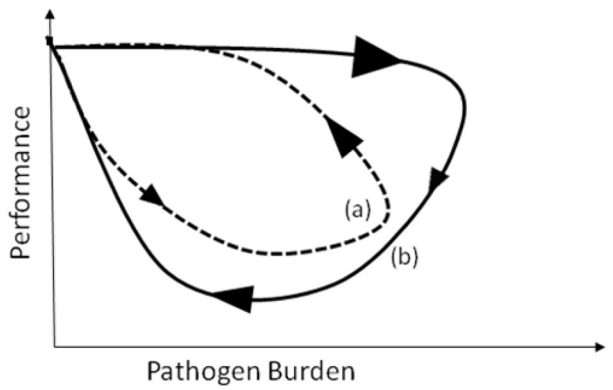

FIGURE 2 | Schematic figure representing pathogen burden–performance trajectories for two individuals (a) and (b) with different resistance and tolerance mechanisms. The direction and size of the arrows indicate the direction and velocity at which trajectories evolve over time.

experienced viral re-activation. Nevertheless its growth rate at the end of the observation period was similar to that at the beginning and remained positive throughout the entire observation period. Furthermore, closer inspection of the trajectory corresponding to pig 1 reveals that reduction in growth was primarily associated with two distinct phases of the infection, i.e., the phase when viraemia levels rapidly increase toward peak levels and the recovery phase when viraemia has almost been cleared. The trajectory thus suggests that the overall impact of infection on performance may be partly associated with pathogen replication at the early stage and may be partly due to immune response mechanisms associated with the recovery process. Pig 2 has initially a similar trajectory to that of pig 1. However, its growth rate increased immediately once viraemia levels started to fall

post peak viraemia and declined again after the re-activation of virus replication. Thus, for pig 2 changes in performance directly mimicked changes in pathogen burden.

As indicated by the arrows in **Figure 1**, trajectories are not only characterized by their shapes but also by the direction and speed at which pathogen burden—performance measures progress over time. These can reveal information about how host resistance and tolerance mechanisms interact during the time course of infection. To illustrate this more clearly, consider the schematic trajectories depicted in **Figure 2**. Both are closed loops indicating full recovery of the host in terms of pathogen elimination and restoration of host performance. Nevertheless, the reverse direction and different sizes of the arrows indicate substantial differences between the hosts in their response to pathogen challenge. For host (a) pathogen burden increases initially slowly (indicated by a small arrow) causing a gradual decrease in performance. This could be considered as moderate resistance and low tolerance at the early stage of the infection. Once pathogen burden peaks, host immune mechanisms counteract pathogen replication and damage leading to fast recovery (indicated by a large arrow). Thus, for host (a) changes in performance are a direct consequence of changes in pathogen burden. In contrast, host (b) experiences initially a rapid increase in pathogen burden (indicated by a large arrow), but its performance is not affected at all during this initial phase of the infection (i.e., low resistance but high tolerance at the early stage of infection). Performance, however, declines after pathogen levels start to decrease, indicating that the immune response rather than the actual pathogens are leading to the reduction in performance. Recovery is initially slow, but accelerates at the later stage of infection when performance levels also recover (i.e., high resistance and high tolerance at the late stage of infection). This situation could arise is different sets of immune mechanisms dominate at different stages of infection, comparing the two hosts.

A NEW CLASSIFICATION OF HOSTS ACCORDING TO THEIR TRAJECTORIES

Trajectories may be considered to illustrate the dynamic interactions of resistance and tolerance mechanisms over time. Thus, a host may be characterized by its trajectory rather than by its resistance and tolerance, which are assumed static. Consequently, instead of aiming to improve host resistance or tolerance, one may aim to achieve an optimal trajectory in the pathogen burden–performance space. This would correspond to breeding for a combined optimal balance of tolerance and resistance mechanisms. But how could this be achieved in practice?

Schneider (2011), after investigating the shapes of "personalized health curves" (equivalent to the pathogen burden–performance trajectories described here) corresponding to various well-studied infections in humans, concluded that most pathogenic and mutualistic host–pathogen interactions can be represented by one of only a few existing archetypical curves. Adapted to the resistance-tolerance context for the majority of livestock diseases, nine major classes of trajectory categories emerge as follows: individual host trajectories may first be classified according to the outcome in terms of infection severity, distinguishing broadly between eventual clearance of pathogens, long-term persistence, and death (illustrated by graphs A, B, C, respectively in **Figure 3**). Within each of these three categories, one may then further classify trajectories according to the long-term impact of infection on host performance. Thus, for infections leading to eventual clearance or long-term persistence, one may distinguish between hosts that experience little or no impact on performance, and those that suffer a temporary or persistent reduction in performance, respectively (illustrated by the different trajectories in **Figures 3A,B**). Similarly, if the final outcome is death, one may categorize trajectories according to whether death was caused either through cumulative damage caused by recurrent episodes of disease outbreaks, each leaving long-term damage, or while clearing the pathogens, or as a direct consequence of uncontrolled pathogen replication. All host–pathogen type interactions will fall within one of these nine categories although the actual shapes of individual trajectories within each category may differ considerably from each other and from those shown in **Figure 3**. Furthermore, depending on the type of pathogens and the host population, only a subset of these nine categories may be realized in practice. In any case, this framework implies that the individual scatter plots produced by data that are inherently noisy and incomplete could be characterized as one of nine types. As a first step in disease control one may thus aim to produce the best feasible trajectory type or look for genotypes associated with specific trajectory types.

HOW TO SUMMARIZE THE TRAJECTORIES BY QUANTITATIVE MEASURES FOR GENETIC ANALYSES?

Having established the potential for pathogen burden–performance trajectories to describe impacts of infection on hosts, we are confronted with a number of further questions. Firstly, how does one generate complete individual trajectories with relatively limited measurements over time? In reality, measurements may only exist for some time points during infection, generating, at best, a fragment of a trajectory. For example, for the pigs in **Figure 1**, growth rates prior to infection were not recorded, making it difficult to infer whether infection led to long-lasting damage in growth. Secondly, is it possible to generate a complete a trajectory based on only few measurements over a restricted time period during an infection? Finally, and most importantly, in cases where all host responses appear to fall within the same trajectory type, how can one quantify individual trajectories and summarize the information into phenotypes for subsequent genetic analyses? The answers to these questions can be found by applying mathematical dynamical systems theory, as outlined below.

DYNAMICAL SYSTEMS THEORY

In mathematical terms, the individual performance *vs*. pathogen burden trajectories may be referred to as trajectories in the *pathogen burden–performance phase space*. Phase space plots, in which individual trajectories are not only characterized by the shape of the curve, but also by the direction and velocity at which these curves evolve over time (indicated by the arrows in **Figures 1–3**), are commonly used to visualize and analyze the global behavior of dynamical systems (Katok and Hasselblatt,

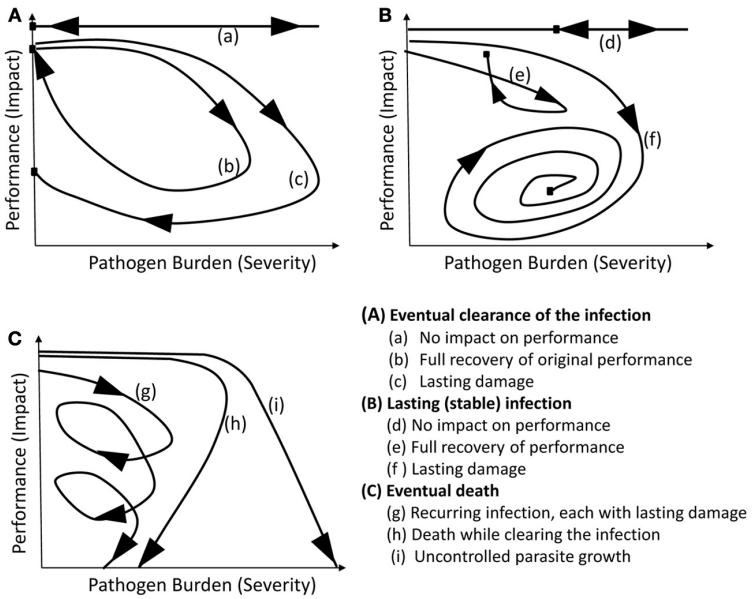

FIGURE 3 | Schematic figure of the nine trajectory archetypes. The three graphs broadly correspond to different resistance categories, while the three individual curves in each graph broadly correspond to different tolerance categories. For further explanation see text.

1995). Indeed, these trajectories elicit system properties that might not otherwise be obvious, such as at what stage the infection is most damaging to the host and whether, when and to what extent recovery occurs.

In order to specify the individual trajectories over the entire time course of infection, and to reduce or remove noise from the data, it is advantageous to use mathematical models fitted to the data rather than the actual data for subsequent analyses. Mathematicians usually describe dynamical system by a set of differential equations. When adopting the dynamical systems theory to the context of host resistance and tolerance, it is therefore necessary to formulate mathematical expressions that describe the change in within-host change in pathogen burden and performance over time based on existing biological knowledge and available data. Thus, step 1 of this approach consists of plotting the (noisy, fragmented) performance *vs*. pathogen burden curves for the individuals in question, and step 2 consists of formulating an appropriate mathematical model that reproduces the essential features of these trajectories. For example, if the data suggest that changes of host performance are partly caused by the pathogen and partly by the immune response, one may start with a three-dimensional system of differential equations that describes changes in host pathogen burden (P) immune response (I) and performance (Y) over time:

$$\begin{aligned}\frac{dP}{dt} &= \mu P - \kappa PI \\ \frac{dI}{dt} &= \lambda + \rho I\frac{P}{P+\phi} - \delta I \\ \frac{dY}{dt} &= -\beta\frac{dP}{dt} - \alpha\frac{dI}{dt}\end{aligned} \tag{7}$$

where μ denotes the replication rate of the pathogen within the host, κ is the rate at which the host immune eliminates the pathogen, λ and δ refer to the replacement and death rate of immune cells, respectively, ρ is the maximum per capita replication rate of the immune response, ϕ represents the parasite density at which the rate of growth of immunity is half maximal, and β and α describe the reduction in performance with respect to changes in pathogen burden and immune response, respectively. System (7) is an extension of the model described by Doeschl-Wilson et al. (2009). Although apparently simple, the model can generate a variety of trajectory types for different combination of model parameters (**Figure 4**).

Having established an appropriate mathematical system that can generate the trajectory types observed from the data, step 3 of the dynamical systems approach consists of analysing the behavior of the mathematical system. In particular, *sensitivity analysis* helps to determine how changes in individual parameter values affect the trajectories and hence helps to identify the key system parameters to be used as phenotypes in subsequent genetic analyses. Bifurcation *theory* can be applied to identify the parameter regions corresponding to the different trajectory types (Steindl and Feichtinger, 2004; Blyuss and Gupta, 2009). Finally, *stability theory* helps to determine the stability of trajectories under small perturbations of the initial conditions (represented, for example, by different challenge doses, different host immunity at the start of the infection, or different performance levels prior to challenge) (Blyuss and Gupta, 2009; Taylor and Carr, 2009). Overall, these techniques will generate a thorough understanding of the trajectories that can be generated in theory and will specify the range of system parameter values corresponding to desired trajectories.

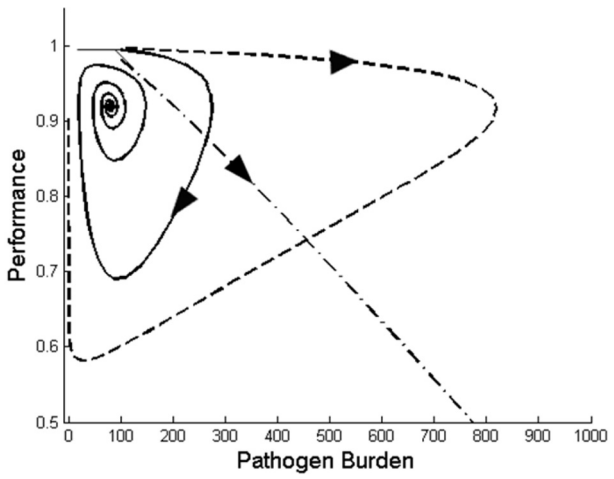

FIGURE 4 | Three different types of performance *vs.* pathogen burden profiles generated by the mathematical dynamical system (7) for different values of the model parameters. The size of the arrows in this graph does not reflect the actual velocity.

The last step in the dynamical systems approach focuses on obtaining values for the model parameters for the population of animals in consideration. This can be achieved by fitting the mathematical model established in step 2 to actual data. Recent advances in Bayesian inference methods (e.g., Savill et al., 2009; Miller et al., 2010) have demonstrated that realistic estimates for system parameters can be obtained from relatively sparse sets of data.

Once estimates for the key system parameters for every individual are obtained, one can use these as phenotypes for conventional quantitative genetic analyses to identify to what extent the trajectories are under host genetic influence. Using model parameters has several benefits over using actual measurements as phenotypes for subsequent analyses. For example, the information provided by many measurements can be summarized into few parameters, measurement error and noise become "averaged out," and with adequate data the corresponding trajectories over time are fully specified, even if available data only relate to a fragment of the full trajectory. Combining results from quantitative genetic analyses with the understanding obtained from the analysis of the system behavior would give new insights into individual impacts of infection on performance. In turn this should help (1) to predict selection responses in terms of infection severity and impact on performance and (2) to identify new informative traits (i.e., the key parameters with a large genetic influence) to be targeted for genetic improvement.

DISCUSSION

Host resistance and tolerance describe the ability of a host to control infection severity and impact on performance. These traits encompass a variety of immune-pathological processes, which are highly dynamic in nature. Thus, the expression of host genetic resistance and tolerance is expected to vary considerably over time. Hitherto, quantitative genetics has treated host resistance and tolerance as static traits. As such, most empirical estimates of both characteristics to date are based on cross-sectional measurements of indicator traits obtained at a specific point in time. However, this static approach applied to dynamic traits not only raises questions about whether the obtained resistance and tolerance parameter estimates are stable over the time course of infection, but also lead to severe estimation bias if field data used as individual records refer to different infection stages across individuals (Bishop et al., 2012; Doeschl-Wilson et al., 2012). In the context of tolerance, cross-sectional measurements impose the additional limitation that a tolerance phenotype can only readily be estimated at the group rather than at the individual level.

Longitudinal measurements of performance and resistance traits (i.e., individual records taken at successive times) are routinely collected for a number of species and diseases (e.g., mastitis or gastro-intestinal parasitism in ruminants). Recording frequency of health traits may even increase if the benefits to livestock production were clearly demonstrated. We have shown in this article that longitudinal measurements provide the opportunity to quantify tolerance at the level of individuals, and may produce new informative phenotypes that describe the interactive effects of host resistance and tolerance over time. In particular, we have shown how, in principle, individual tolerance estimates could be obtained within the conventional random regression framework, and introduced a non-conventional dynamical systems approach to generate new phenotypes describing impacts of infectious pathogens on hosts. The pros and cons of these approaches will be discussed below, but it should first be noted that both approaches rely on the following conditions:

- Both host performance and pathogen burden can be measured repeatedly over the time period under consideration for each individual.
- The performance trait is such that the phenotype for individuals in the absence of pathogen challenge is either known or can be inferred for the time period over which tolerance is estimated.
- Other factors influencing performance throughout the time period of infection, in addition to pathogen challenge, can be properly accounted for.

Random regression models have proved to be a powerful tool for quantitative genetic analyses, not only for linear models but also for measures that represent curves (Meyer and Kirkpatrick, 2005). Such models are particularly attractive for quantitative genetic analyses of host resistance and tolerance as they can be readily accommodated in the conventional multivariate linear mixed model framework of quantitative genetics. Consequently, established methods can be used for estimating genetic parameters associated with host resistance and tolerance, and for genetic evaluations. However, in addition to a requirement for large datasets, a major potential drawback of using random regression models for tolerance is that these models require an explicit mathematical expression describing the relationship between host performance and pathogen burden. Meyer and Kirkpatrick (2005) demonstrated that the random regression approach can be applied to a

wide range of non-linear functions, as long as the model is linear in the regression coefficients. However, as both host performance and pathogen burden vary over time and are mediated by the host immune response, it may not always be possible to express this relationship by a suitable function. In fact, even the relatively simple model described by Equations (5) and (6), points to two major issues that may need to be dealt with: firstly, the independent variable, pathogen burden itself is a function of time, and may change (increase, decrease, or both) over time. The independent variables of random regression models are however typically continuous and strictly monotonic (e.g., time, age, etc.). Secondly, the dynamic systems approach revealed that the relationship between host performance and pathogen burden over the time course of infection may not be representable as a mathematical function, but rather as a mathematical relation. Mathematical functions are a subset of relations for which every value of an independent variable (here pathogen burden) corresponds to a unique value of the dependent variable (here performance). As illustrated by the trajectories in **Figure 1**, this may not be the case as a particular value of pathogen burden may correspond to two or more performance trait values. The properties and applicability of the random regression approach has yet to be fully investigated.

The trajectory approach relaxes the stringent assumption of random regression models that the relationship between host performance and pathogen burden can be adequately represented by a mathematical function. Instead, a simple classification of the data trajectories into one of nine categories may provide a useful categorical phenotype for subsequent genetic studies. Furthermore, trajectories would enable the introduction of powerful tools from dynamical systems theory into quantitative genetics. According to dynamical systems theory, performance is related to pathogen burden by a system of differential equations that describes the dynamic host–pathogen interactions affecting infection severity and impact. The close affinity of the system's parameters to the underlying biological processes makes these parameters attractive phenotypes for subsequent genetic analyses. Although rarely used in quantitative genetic analyses, differential equation models appear a promising tool for handling dynamic processes within the conventional quantitative genetics framework. Integration of these more complex mathematical models into genetic analyses, as outlined by the different steps above, may enhance our understanding of the key processes that determine infection severity and reduction in performance and simultaneously provide valuable insight about the host genetic influence on these.

Dynamical systems theory is well-established in mathematics for analyzing the full spectrum of patterns produced by a complex dynamical system. They have proved useful to study infection processes within individual hosts and in populations (e.g., Blyuss and Gupta, 2009; Taylor and Carr, 2009). In the context of genetic improvement programmes, dynamical systems theory would not only provide new phenotypes, but also help to specify potential improvement targets. In particular, it can be used to determine which types of pathogen burden–performance trajectories could be produced in principle and how much shift (i.e., genetic gain) in the parameter values is required to achieve desirable trajectories.

As outlined above, the individual building blocks needed for adopting resistance-tolerance trajectories into breeding programmes already exist. There are, however, several challenges associated with the dynamical systems approach, of which perhaps the biggest one is to identify an appropriate model that reproduces the essential features observed from the data. There is a vast literature on dynamic host–pathogen interaction models [see e.g., reviews by Louzoun (2007); Mata and Cohn (2007); Doeschl-Wilson (2011)], ranging from simple models such as the one presented here [Equation (7)], where pathogens and immune response are summarized as single entities (e.g., Antia et al., 1996; Restif and Koella, 2004; Doeschl-Wilson et al., 2009), to highly complex models comprising a large number of differential equations with many parameters (e.g., Marchuck et al., 1991; Kosmrlj et al., 2010). For the purpose of quantitative genetic analyses, simple models requiring fewer parameters and thus giving rise to fewer phenotypic traits are more attractive than complex models. Nevertheless, it remains to be tested whether a relatively simple model can adequately reproduce the main features of the pathogen burden–performance trajectories emerging from the data. Furthermore, if an appropriate model can be identified, the next challenge that arises is to fit the model to existing data. Bayesian methods have proved powerful in providing reliable parameter estimates for differential equation models (e.g., Girolami, 2008; Savill et al., 2009; Miller et al., 2010), but to the best of our knowledge have not been applied to the large data sets needed for quantitative genetic analyses. Further optimization of the computational algorithms may be necessary for efficiently handling the large amount of data usually required for genetic analyses.

Finally, it should be noted that the trajectory approach can be adapted to field data, as the methodology doesn't require individuals to be at the same stage of infection nor does it require prior knowledge of the time of onset of infection. This approach may thus provide a means to capture tolerance of animals to infections under natural rather than experimental pathogen challenge, which has been difficult up to now (Doeschl-Wilson et al., 2012).

CONCLUSIONS

Host genetic resistance and tolerance to infectious pathogens are highly desirable targets for genetic improvement. Up to now genetic analysis of host tolerance has been hindered by the lack of appropriate methods to obtain reliable tolerance phenotypes, in particularly at the level of individuals. We have outlined two alternative approaches to fill this gap, a statistical random regression approach and a mathematical dynamical systems approach. We have shown that random regression models provide a means of extending the methodology of quantifying group tolerance to the individual level. However, application of these models in practice comes with strict data requirements and depends on whether the relationship between within-host pathogen burden and performance can be adequately represented by a mathematical model that is linear in its regression coefficients. Mathematical dynamical systems theory offers a promising alternative to the statistical models currently used in quantitative genetics, as it captures the dynamic interaction of host resistance and tolerance mechanisms throughout the infection and provides

static parameters amenable for genetic analyses. It builds upon performance-pathogen burden trajectories that can be derived from repeated pairwise observations of host performance and pathogen burden over the time course of the infection. Future studies are warranted to test the theoretical concepts introduced here with simulated and real data.

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REFERENCES

Antia, R., Koella, J. C., and Perrot, V. (1996). Models of the within-host dynamics of persistent mycobacterial infections. *Proc. Biol. Sci.* 263, 257–263.

Ayres, J. S., and Schneider, D. S. (2012). Tolerance of infections. *Annu. Rev. Immunol.* 30, 271–294.

Bishop, S. C., Doeschl-Wilson, A. B., and Woolliams, J. A. (2012). Uses and implications of field disease data for livestock genomic and genetics studies. *Front. Gene.* 3:114. doi: 10.3389/fgene.2012.00114

Bishop, S. C., and Woolliams, J. A. (2010). On the genetic interpretation of disease data. *PLoS ONE* 5:e8940. doi: 10.1371/journal.pone.0008940

Blyuss, K. B., and Gupta, S. (2009). Stability and bifurcations in a model of antigenic variation in malaria. *J. Math. Biol.* 58, 923–937.

Coop, R. L., and Kyriazakis, I. (1999). Nutrition–parasite interactions. *Vet. Parasitol.* 84, 187–204.

Doeschl-Wilson, A. B. (2011). The role of mathematical models of host-pathogen interactions for livestock health and production - a review. *Animal* 5, 895–910.

Doeschl-Wilson, A. B., Brindle, W., Emmans, G., and Kyriazakis, I. (2009). Unravelling the relationship between animal growth and immune response during micro-parasitic infections. *PLoS ONE*, 4:e7508. doi: 10.1371/journal.pone.0007508

Doeschl-Wilson, A. B., Villanueva, B., and Kyriazakis, I. (2012). The first step towards genetic selection for host tolerance to infectious pathogens: obtaining the tolerance phenotype through group estimates. *Front. Gene.* 3:265. doi: 10.3389/fgene.2012.00265

Falconer, D. S., and Mackay, T. F. C. (1996). *Introduction to Quantitative Genetics, 4th Edn.* UK: Longman.

Girolami, M. (2008). Bayesian inference for differential equations. *Theor. Comput. Sci.* 408, 4–16.

Glass, E. J. (2012). The molecular pathways underlying host resistance and tolerance to pathogens. *Front. Gene.* 3:263. doi: 10.3389/fgene.2012.00263

Katok, A., and Hasselblatt, B. (1995). *Introduction to the Modern Theory of Dynamical Systems. Encyclopedia of Mathematics and Its Applications*. UK: Cambridge University Press, 54.

Kause, A. (2011). Genetic analysis of tolerance to infections using random regressions: a simulation study. *Genet. Res.* 93, 291–302.

Kause, A., and Ødegård, J. (2012). The genetic analysis of tolerance to infections: a review. *Front. Gene.* 3:262. doi: 10.3389/fgene.2012.00262

Kosmrlj, A., Read, E. L., Qi, Y., Allen, T. M., Altfeld, M., Deeks, S. G., et al. (2010). Effects of thymic selection of the T-cell repertoire on HLA class I-associated control of HIV infection. *Nature* 465, 350–354.

Louzoun, Y. (2007). The evolution of mathematical immunology. *Immunol. Rev.* 216, 9–20.

Marchuck, G. I., Petrov, R. V., Romanyukha, A. A., and Bocharov, G. A. (1991). Mathematical model of antiviral immune response. I., data, analysis, generalized picture construction and parameter evaluation for Hepatitis, B. *J. Theor. Biol.* 151, 1–40.

Mata, J., and Cohn, J. (2007). Quantitative modeling of immune responses. *Immunol. Rev.* 216, 5–8.

Mauricio, R., Rausher, M. D., and Burdick, D. S. (1997). Variation in the defense strategies of plants: are resistance and tolerance mutually exclusive? *Ecology* 78, 1301–1311.

Meyer, K., and Kirkpatrick, M. (2005). Up hill down dale: quantitative genetics of curvaceous traits. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 360, 1443–1455.

Miller, M. R., Råberg, L., Read, A. F., and Savill, N. J. (2010). Quantitative analysis of immune response and erythropoiesis during rodent malaria infection. *PLoS Comput. Biol.* 6:e1000946. doi: 10.1371/journal.pcbi.1000946

Råberg, L., Graham, A. L., and Read, A. F. (2009). Decomposing health: tolerance and resistance to parasites in animals. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 364, 37–49.

Råberg, L., Sim, D., and Read, A. F. (2007). Disentangling genetic variation for resistance and tolerance to infectious disease in animals. *Science* 318, 318–320.

Restif, O., and Koella, J. (2004). Concurrent evolution of resistance and tolerance to pathogens. *Am. Nat.* 164, E90–E102.

Rowland, R., Lunney, J., and Dekkers, J. (2012). Control of porcine reproductive and respiratory syndrome (PRRS) through genetic improvements in disease resistance and tolerance. *Front. Gene.* 3:260. doi: 10.3389/fgene.2012.00260

Savill, N. J., Chadwick, W., and Reece, S. E. (2009). Quantitative analysis of mechanisms that govern red blood cell age structure and dynamics during anaemia. *PLoS Comput. Biol.* 5:e1000416. doi: 10.1371/journal.pcbi.1000416

Schneider, D. (2011). Tracing personalized health curves during infections. *PLoS Biol.* 9:e1001158. doi: 10.1371/journal.pbio.1001158

Schneider, D. S., and Ayres, J. S. (2008). Two ways to survive infection: what resistance and tolerance can teach us about treating infectious diseases. *Nat. Rev. Immunol.* 8, 889–895.

Simms, E. L. (2000). Defining tolerance as a norm of reaction. *Evol. Ecol.* 14, 563–570.

Simms, E. L., and Triplett, J. (1994). Costs and benefits of plant responses to disease-resistance and tolerance. *Evolution* 48, 1973–1985.

Steindl, A., and Feichtinger, G. (2004). Bifurcations to periodic solutions in a production/inventory model. *J. Nonlin. Sci.* 14, 469–503.

Taylor, M. L., and Carr, T. W. (2009). An SIR epidemic model with partial temporary immunity modelled with delay. *J. Theor. Biol.* 59, 841–880.

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The genetic analysis of tolerance to infections: a review

Antti Kause[1]* and Jørgen Ødegård[2]

[1] Biotechnology and Food Research, Biometrical Genetics, MTT Agrifood Research Finland, Jokioinen, Finland
[2] Nofima, Ås, Norway

Edited by:
Andrea B. Doeschl-Wilson, The University of Edinburgh, UK

Reviewed by:
Mairead L. Bermingham, The University of Edinburgh, UK
Sunday Peters, Cornell University, USA

***Correspondence:**
Antti Kause, Biotechnology and Food Research, Biometrical Genetics, MTT Agrifood Research Finland, FI-31600, Jokioinen, Finland.
e-mail: antti.kause@mtt.fi

Tolerance to infections is defined as the ability of a host to limit the impact of a given pathogen burden on host performance. Uncoupling resistance and tolerance is a challenge, and there is a need to be able to separate them using specific trait recording or statistical methods. We present three statistical methods that can be used to investigate genetics of tolerance-related traits. Firstly, using random regressions, tolerance can be analyzed as a reaction norm slope in which host performance (*y*-axis) is regressed against an increasing pathogen burden (*x*-axis). Genetic variance in tolerance slopes is the genetic variance for tolerance. Variation in tolerance can induce genotype re-ranking and changes in genetic and phenotypic variation in host performance along the pathogen burden trajectory, contributing to environment-dependent genetic responses to selection. Such genotype-by-environment interactions can be quantified by combining random regressions and covariance functions. To apply random regressions, pathogen burden of individuals needs to be recorded. Secondly, when pathogen burden is not recorded, the cure model for time-until-death data allows separating two traits, susceptibility and endurance. Susceptibility is whether or not an individual was susceptible to an infection, whereas endurance denotes how long time it took until the infection killed a susceptible animal (influenced by tolerance). Thirdly, the normal mixture model can be used to classify continuously distributed host performance, such as growth rate, into different sub-classes (e.g., non-infected and infected), which allows estimation of host performance reduction specific to infected individuals. Moreover, genetics of host performance can be analyzed separately in healthy and affected animals, even in the absence of pathogen burden and survival data. These methods provide novel tools to increase our understanding on the impact of parasites, pathogens, and production diseases on host traits.

Keywords: cure model, genotype-by-environment interaction, mixture model, quantitative genetics, random regression, resistance, statistical methods, tolerance

INTRODUCTION

Tolerance and resistance are two different defense mechanisms to defend against pathogens and parasites. Resistance is the ability of a host to prevent pathogen entry and to control pathogen life cycle in a way to reduce pathogen burden within a host individual. Tolerance to infections, in turn, is defined as the ability of the host to limit the impact of a given pathogen burden on host health, performance, and ultimately on host fitness (Cluniess-Ross, 1932; Painter, 1958; Albers et al., 1987; Simms and Triplett, 1994; Simms, 2000) (**Figure 1**).

Being able to uncouple resistance and tolerance is essential for several reasons. Firstly, they have different impact on the arms-race co-evolution between the host and the pathogen (Mauricio et al., 1997; Rausher, 2001; Bishop and MacKenzie, 2003; Best et al., 2008). Moreover, both in animals and plants, tolerance and resistance are weakly genetically correlated, and thus they are genetically different traits (Leimu and Koricheva, 2006; Ødegård et al., 2011b; Kause et al., 2012). Finally, animal and plant breeders should exploit both increased resistance and tolerance to ensure global food security.

In addition to pathogens, tolerance can be assessed against abiotic factors such as temperature, heavy metals, or against production diseases causing damage to body tissues (Ravagnolo and Misztal, 2000a,b; Schat et al., 2002; Bloemhof et al., 2012; Kause et al., 2012). Naturally, production diseases, such as ascites, are not standard disease traits caused by a pathogen or parasite infection. Thus, there is no co-evolution between a host and a production disease, and the production disease does not evolve in response to the evolution of the host. Nevertheless, improved resistance and tolerance can be both used to reduce the harmful effects of production diseases on farmed animals, motivating their tolerance analysis (Kause et al., 2012). From hereon in this paper, pathogen burden is used as a general term to refer to a pathogen load of an individual, for instance, number or biomass of ecto- and endoparasites, number of pathogens in a blood sample, or severity of a production disease. In plants, pathogen burden may refer to the biomass or number of herbivores, or percentage of leaf area lost to herbivores.

The objective of this paper is to present recent statistical advances in the genetic analysis of tolerance-related traits. Firstly, random regression models have been applied to

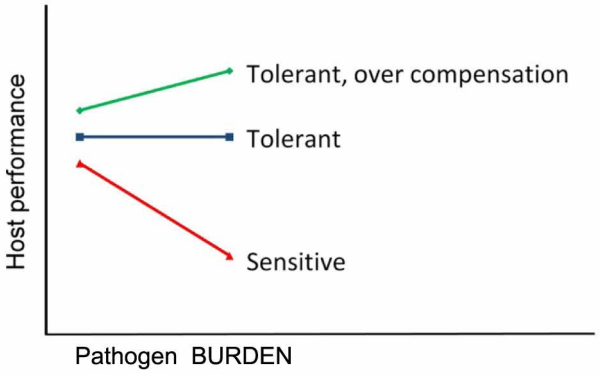

FIGURE 1 | Tolerance to infections. Tolerance is the reaction norm slope of host performance regressed against individual's pathogen burden. The lines represent performance of three genotypes with a different degree of tolerance.

Box 1 | A random regression model.

An animal model random regression model is of the form:

$y_i = b_0 + b_1 PBurden + b_{0i} + b_{1i} PBurden_i + \epsilon_i$, where y_i is host performance of an individual i at its pathogen burden $PBurden$, b_0 is the fixed population mean intercept, b_1 $PBurden$ is the fixed population mean tolerance slope, b_{0i} is the random genetic effect of intercept for an individual i, b_{1i}PBurden$_i$ is the random genetic effect of tolerance slope for an individual i, and ϵ_i is the random error term. Both b_{0i} and b_{1i} are modeled with a pedigree, allowing the estimation of their genetic variance.

Covariance functions. Genetic variance of host performance as a function of pathogen burden can be calculated: as $x'_{PBurden}\mathbf{G}x_{PBurden}$, where $\mathbf{G} = \begin{bmatrix} \sigma^2_{b0} & \sigma_{b0b1} \\ \sigma_{b0b1} & \sigma^2_{b1} \end{bmatrix}$, σ^2_{b0} and σ^2_{b1} are genetic variances for intercept and slope, respectively, and σ_{b0b1} is covariance between the two terms (Kolmodin and Bijma, 2004). The term $x_{PBurden}$ is a vector [1 $PBurden$]′ in which $PBurden$ refers to a pathogen burden value on the x-axis. A genetic correlation between the performance of non-infected ($PBurden = 0$) and infected individuals at a certain $PBurden$ value can be calculated as: $r_G = \frac{x'_0 \mathbf{G} x_{PBurden}}{\sqrt{x'_0 \mathbf{G} x_0 \times x'_{PBurden} \mathbf{G} x_{PBurden}}}$, where $\mathbf{G}$ is the genetic (co)variance matrix of slope and intercept, x_0 is a vector of [1 0]′, and $x_{PBurden}$ is as described earlier (Calus et al., 2004).

tolerance analysis. They allow a sophisticated genetic analysis of traits defined as functions as well as the quantification of genotype-by-environment interactions (G × E) induced by infections (Kause, 2011; Kause et al., 2012). Secondly, Ødegård et al. (2011b,c) introduced a cure model to separate "susceptibility" and "endurance" from challenge test data with time-until-death observations (without having any knowledge about infection status of the animals). The first trait is comparable to resistance, while endurance may be influenced by tolerance. "Susceptibility" can be defined as whether or not the animal is liable to die as a result of an infection (i.e., long-term survival, which is likely associated with resistance), while "endurance" is defined as how long time it takes before a potential infection kills the animal (which is likely associated with tolerance). Both endurance and susceptibility may show genetic variation, and may be viewed as different genetic factors affecting survival under an infection. Finally, normal mixture models can be extended to involve responses in host performance traits (e.g., growth curves) specific to healthy and affected individuals (Wang and Bodner, 2007; Madsen et al., 2008).

RANDOM REGRESSION MODELS

Tolerance is by definition the change in host performance as a function of pathogen burden (Simms, 2000), and hence, it is natural to apply random regression models to estimate genetic parameters and breeding values for tolerance (Kause, 2011). Using random regressions, tolerance can be analyzed as a reaction norm in which host performance (on y-axis) is regressed against pathogen burden of individuals (on x-axis) (**Box 1**). It is important to note that pathogen burden is measured separately from each individual, and it is not a general environmental characteristic. The slope of such a regression is consistent with the definition of tolerance (**Figure 1**), and hence genetic variance in regression slopes is the genetic variance for tolerance (Kause, 2011).

The intercept of the tolerance regression is interpreted as the host performance in a pathogen-free environment, and the genetic correlation between the slope and the intercept quantifies the degree to which host performance under no infection is genetically traded off with tolerance. Moreover, genetic correlations of the slope and intercept with third-party traits can be estimated by extending the random regression model to multitrait animal or sire model (Kause et al., 2012).

In animals, pathogen burden is typically a continuously distributed trait, especially when a population is under a natural pathogen infection (Stear et al., 1995; Kuukka-Anttila et al., 2010). Even in a challenge test in which all individuals are exposed to the same initial pathogen load, variation among individuals in resistance creates continuous variation in pathogen burden. Random regression models allow genetic analysis of tolerance along a continuous pathogen burden trajectory. In animal breeding, random regression models have been commonly applied to the reaction norm analysis of G × E (Henderson, 1982; Meyer and Hill, 1997; Calus et al., 2004; Schaeffer, 2004; Lillehammer et al., 2009).

TOLERANCE-INDUCED VARIATION IN HOST PERFORMANCE

Genetic variation in tolerance may induce G × E in host performance, leading to changes in genetic variation of host performance along an increasing pathogen burden trajectory. For instance, in **Figure 1**, genetic variance in host performance is elevated along increased pathogen burden due to diverging tolerance reaction norms. In poultry, pigs, and aquaculture species, breeding nucleuses may be held infection-free due to biosecurity reasons, whereas commercial production and/or collection of sib and progeny information for breeding value estimation occurs at field farms with diverse diseases present. Such a design may induce G × E due to variation in the level of tolerance, which should be accounted for in breeding value evaluations.

In an infection-free environment, individual variation in host performance, e.g., in growth rate, is due to variation in genetic

potential for growth and unexplained environmental variation. Under infection, in turn, individual variation in both resistance and tolerance induce additional variation into host performance. Some individuals are fully resistant or are not exposed to an infection, and thus their growth is not influenced by the infection. Some individuals are infected, and the degree to which their growth rate is reduced depends on their pathogen burden and the level of tolerance. Growth of fully tolerant individuals is not affected, whereas growth of very sensitive ones is greatly reduced.

Despite the large number of studies dealing with the changes induced by biotic (e.g., diet) and abiotic factors in general (Hoffmann and Merilä, 1999; Kause and Morin, 2001; Charmantier and Garant, 2005), there has been only a limited focus on infection-induced changes in genetic parameters and the consequent environment-specific genetic responses to selection (van der Waaij et al., 2000). Infections are indeed known to induce changes in heritability of host performance traits (Charmantier et al., 2004; Pakdel et al., 2005; Zerehdaran et al., 2006; Kause et al., 2007, 2012; Vehviläinen et al., 2008; Lewis et al., 2009). Yet, currently we do not know how much of the phenotypic variation in host performance is in fact created by infections and the associated tolerance. A study by Kause et al. (2012) showed that coefficient of phenotypic variation in broiler body weight was elevated from 11.5% when birds were healthy, to 19.1% when birds were severely affected by ascites. Similarly, coefficient of genetic variation was increased from 4.9% to 7.9%, implying the changes in variance can be extensive (**Figure 2**). It is hypothesized that in populations exposed to infections, a large proportion of phenotypic variance in host traits is induced by infections and the associated individual variation in resistance and tolerance.

Random regression models combined with covariance functions (Kirkpatrick et al., 1990; Meyer and Hill, 1997) provide means to quantify the changes in phenotypic and genetic variances in host traits along a continuous pathogen burden trajectory (Kause, 2011; Kause et al., 2012). Given the genetic (co)variance estimates of tolerance slope and intercept estimated using random regressions, the changes in genetic variance in host performance can be calculated using formulas (**Box 1**; **Figure 2**).

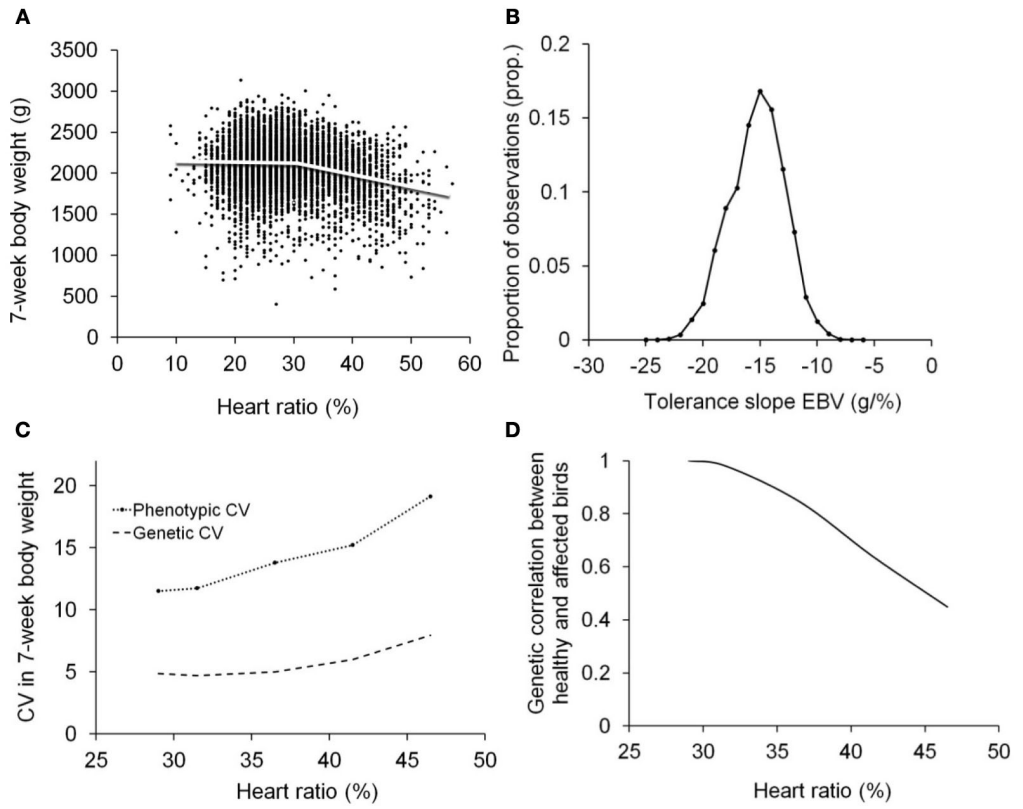

FIGURE 2 | Tolerance analysis using random regressions and covariance functions illustrated using data on 7-week body weight and heart ratio of broilers [reproduced from Kause et al. (2012); http://creativecommons.org/licenses/by/3.0/]. Heart ratio, the ratio of right ventricular weight to total heart weight, is an indicator of ascites resistance, the birds with higher than 27–30% heart ratio typically being ascitic (Wideman et al., 1998). Tolerance is the change in body weight as a function of increasing ascites severity, measured as the hearth ratio. **(A)** Population average tolerance curve with body weight on *y*-axis and ascites severity on *x*-axis. **(B)** Frequency distribution of estimated breeding values for tolerance slopes of individuals, showing sensitive (steep negative slope) and more tolerant (weak negative slope) genotypes. **(C)** Increased coefficients of phenotypic and genetic variation in body weight as a function of ascites severity, showing ascites molds trait variation. **(D)** Genetic correlation between healthy birds and birds with different degree of ascites severity, showing ascites creates genotype re-ranking (Kause et al., 2012).

The same logic can be applied to the maternal and environmental components of (co)variance.

TOLERANCE-INDUCED GENOTYPE RE-RANKING IN HOST PERFORMANCE

Crossing tolerance reaction norms create genotype re-ranking in host performance traits across pathogen burden trajectory. This is similar to any genotype re-ranking across environmental gradients (Via and Lande, 1985), with the difference that now the environment is pathogen burden of individuals (Kause et al., 2012). The two forms of G × E, scaling effect and genotype re-ranking, facilitate environment-dependent genetic responses, yet the re-ranking is more severe issue for selective breeding because genotypes in one environment are not necessarily the best ones in the other environments. Re-ranking across environments can be quantified by a genetic correlation between measurements in two environments for a given trait (Falconer, 1952).

The degree of re-ranking between any two pathogen burden levels can be calculated by combining random regression results with covariance functions (**Box 1**). For instance, ascites induced moderate genotype re-ranking in broiler body weight, the genetic correlation of healthy birds with weakly affected birds being unity but with severely affected birds 0.45 (Kause et al., 2012; **Figure 2**). In field data sets with multiple environments, infection pressure is typically not the only environmental factor varying across environments, yet the effect of pathogen burden on G × E could be revealed using a combined reaction norm and multi-trait model (Windig et al., 2011), in which pathogen burden is modeled as a continuous reaction norm and the discrete environments capturing other environmental factors are modeled as separate discrete traits. Performing extensive infection-challenge tests is impractical in many farm animal species, but the combined reaction norm and multi-trait model may be an effective additional method for revealing the degree of G × E induced by infections.

Infections do not induce only genotype re-ranking and a change in variance but also changes in the correlation structure of resistance, growth, and reproduction traits (de Greef et al., 2001; Kause et al., 2005, 2012; Zerehdaran et al., 2006; Kuukka-Anttila et al., 2010). The modification of genetic architecture of host traits by pathogens, parasites, and production diseases, mediated by tolerance genetics, may play a more fundamental role in animal breeding and microevolution than has been previously thought.

DATA REQUIREMENTS FOR RANDOM REGRESSION

Obtaining a solid *x*-axis is a major challenge for the tolerance analysis in animals because the *x*-axis should consists of individual-level quantitative data on pathogen burden (e.g., number of parasites, pathogen biomass). Qualitative data on burden (infected vs. non-infected individuals) creates biased estimates of genetic variance for tolerance (Kause, 2011). Moreover, if the *x*-axis consists of the average burdens of each environment, rather than individual-level burden measurements, then high host performance of a genotype at a given pathogen burden can be a result of high resistance and/or high tolerance, impeding a proper tolerance analysis. The analyzed host performance trait, in turn, can be feed intake, growth, reproduction, survival or a physiological trait, which together can be used to reveal mechanisms contributing to variation among genotypes in tolerance.

A split-family design with both an infection-free control and an experimental challenge test is the most effective design for tolerance analysis. In this way, infected animals are a random sample of their family and thus there will be a real causal relationship between host performance and pathogen burden (Tiffin and Inouye, 2000; Kause, 2011). This requires, however, that all the challenged individuals get the same pathogen burden level. This rarely is the case because individuals have innate individual variation in resistance, creating variation in pathogen burden even in a challenge test. Variation in resistance can be potentially related to the host performance traits used on the *y*-axis in the tolerance regression, biasing the estimate of genetic variation for tolerance (Tiffin and Inouye, 2000; Kause, 2011). As an alternative to the control-and-challenge test design, all individuals can be first recorded under infection-free conditions (e.g., for mature body weight), and then re-recorded after experimental exposition to equal pathogen burden level. However, such an analysis is unjustified in cases in which host performance shows natural temporal variation (e.g., variation in growth curves), which is thus confounded with tolerance (Albers et al., 1987; Bisset and Morris, 1996; Woolaston and Windon, 2001). Trypanotolerance of African cattle has been analyzed as a change in body weight in response to an experimental infection by *Trypanosoma congolense*, but although the number of parasites in the blood of individuals was recorded, it was not used to standardize the host performance changes of individuals (Hanotte et al., 2003; van der Waaij et al., 2003).

Under naturally occurring infection, it is possible that either high (or low) performing individuals are infected, leading to biased estimates of genetic variation for tolerance (Tiffin and Inouye, 2000; Kause, 2011). This is a major weakness of field data sets, because it is well established that individuals with initially different growth or life-history trait levels may be differently exposed to infections, parasites and production diseases (Arendt, 1997; Rauw et al., 1998), confounding the cause-and-effect relation between pathogen burden and reduction in host performance.

Random regression models require large sample sizes, e.g., within sire families. Decrease in family size leads to upward-biased genetic variance estimates for tolerance slope (Kause, 2011). This can be illustrated in a sire model set up. When a small number of individuals are sampled for each sire family, the sample is no longer representative of the true distribution and single observations have strong impact on the slope estimate. For some families the slope is underestimated, for others overestimated, and thus genetic variance estimate for slope is artificially increased. With heritability of 0.3 for tolerance slope, more than 50 sibs per family are required to obtain unbiased estimates of slope variance using a sire model analysis (Kause, 2011). Moreover, genetic correlation between tolerance slope and intercept is easily biased downward when family size is low. An upward (downward) bias in the slope of a family pushes the intercept downward (upward), creating an artificial negative genetic trade-off when it does not exist in reality. This can be avoided by using high family sizes and

high number of non-infected individuals that force the intercept of a genotype to be placed close to the real value (Mauricio et al., 1997; Kause, 2011).

When each host individual has only a single performance record, it is possible to estimate genetic variance and breeding values for tolerance slope, but not its residual variance. Heritabilities for tolerance slope can be estimated when each individual has several performance observations, e.g., the initial performance under conditions of no infection and thereafter the performance after an infection. By using regression slopes of individuals as raw observations in the genetic analysis, both environmental and genetic components of slope variance and heritability can be estimated (Schaeffer, 2004).

Random regression can be applied to non-linear reaction norms (Kirkpatrick et al., 1990; Meyer and Hill, 1997; Schaeffer, 2004) and plateau-linear regression models (Ravagnolo and Misztal, 2000a,b; Kause et al., 2012), and thus the impact of pathogens on host performance does not need to be analyzed as a linear relationship.

A CURE MODEL FOR TIME-UNTIL-DEATH DATA

The random regression approach requires individual-level data on pathogen burden which may be challenging to record. The cure model for time-until-death data provides a possibility to analyze genetics of resistance (or susceptibility) and endurance without a need for pathogen burden recording.

Many studies, especially on aquaculture species, have analyzed survival or time-until-death in a challenge test in which individuals are experimentally exposed to a specific pathogen (Ødegård et al., 2011a). Moreover, survival analysis has been applied to time-until-death data when mortality factors remain unknown (e.g., Ducrocq and Casella, 1996; Serenius and Stalder, 2004; Vehviläinen et al., 2010). A typical assumption in such analyses is that individuals with high probability of survival are resistant. However, an individual can survive if it has either high resistance, or low resistance but high tolerance (**Figure 3**), or was never exposed to a pathogen. The cure survival models are used for modeling of time-until-death data which include a fraction of non-susceptible animals, i.e., animals that are not liable to die as a result of the infection (Farewell, 1982). Ødegård et al. (2011c) developed a cure model aiming to distinguish two traits, "susceptibility" and "endurance," from time-until-death data. These two concepts may be comparable with resistance and tolerance.

In a survival analysis the infection status of each animal is typically unknown. Under pathogen attack, some animals may be fully capable of avoiding death (non-susceptible), either by resisting the infection, or by a successful recovery after the initial infection due to high tolerance (**Figure 3**). Furthermore, the degree of tolerance may also vary among the susceptible individuals, potentially causing variation in their expected time-until-death. As mortality is usually recorded over a limited follow-up period, a fraction of susceptible animals are also likely to be alive at the time of recording. For susceptible animals, the ability to survive depends on the expected time-until-death of the animal, which may show genetic variation. Hence, analogy of the terms "endurance" and "susceptibility" with tolerance and resistance are not necessarily clear-cut in a survival analysis, due to the fact that

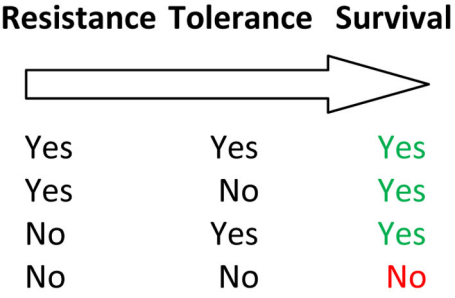

FIGURE 3 | Contribution of resistance and tolerance to mortality due to a specific pathogen. Only individuals without resistance and tolerance will eventually die given a sufficiently long follow-up period. When having a limited follow-up period, individuals with high tolerance may still be alive at the end of an experiment.

one only observes the extreme outcomes of an infection (whether or not an animal dies). Although "endurance" and "susceptibility" are impossible to separate on individual survivors, these two factors may still be distinguished on a family level using longitudinal survival analysis (i.e., short-term mortality rates vs. long-term survival).

A classical survival analysis of time-until-death assumes that all individuals are at risk and that all will eventually die given a sufficiently long follow-up period. When studying lifespan in general this is necessarily true, but may not hold when testing for mortality due to a specific pathogen. For non-susceptible animals time-until-death will necessarily be censored, irrespective of the follow-up time, and survival time may thus be a poor indicator for specific pathogen resistance. The endurance reflects the expected mortality per time-unit among susceptible individuals, but will have no effect on survival of the non-susceptible individuals (Farewell, 1982).

The survivors are likely a mixture of non-susceptible long-term survivors and a fraction of susceptible (but highly endure) animals being still alive, and the true condition of each animal is unknown (unless the animal dies). In the cure model, probabilities of the alternative settings (non-susceptible or susceptible but still alive) can be estimated while simultaneously taking into account variation in endurance among the surviving animals (Ødegård et al., 2011c; **Box 2**).

The cure model has been applied to time-until-death data in farmed shrimp challenge-tested with the Taura syndrome virus (Ødegård et al., 2011b). It was estimated that although 72% of the shrimp survived, only 62% could be considered non-susceptible. The underlying heritability (±SE) for susceptibility was high (0.41 ± 0.07), while the heritability of endurance was low, albeit significant (0.07 ± 0.03). The most striking result was that endurance and susceptibility were seemingly distinct genetic traits ($r_G = 0.22 \pm 0.25$). The low genetic variation for endurance and the genetic independency of endurance and susceptibility are in line with the results on other animal species (Kause et al., 2012). These results have substantial impact on how disease challenge-testing should be performed. If the aim is to improve long-term survival under an infection pressure,

Box 2 | A cure model.

In a mixed population of susceptible ($z = 1$) and non-susceptible ($z = 0$) animals the probability for an individual being still alive (censored) ($c = 0$) at time t is:

$$\begin{aligned} Pr(c = 0|t) &= Pr(c = 0|t,\ z = 1)Pr(z = 1) \\ &+ Pr(c = 0|t, z = 0)Pr(z = 0) \\ &= Pr(c = 0|t,\ z = 1)Pr(z = 1) + \big(1 - Pr(z = 1)\big), \end{aligned}$$

where $Pr(z = 1)$ is the prior probability of being susceptible and $Pr(c = 0|t, z = 0) = 1$. The probability of being still alive for susceptible animals, $Pr(c = 0|t,\ z = 1)$, is a function of the endurance of the animal. Furthermore, if survival time is split into a series of binary survival scores (e.g., s_1 to s_t, where 0 indicates survival), this probability is:

$$Pr(c = 0|t,\ z = 1) = \prod_{j=1}^{t} Pr(s_j = 0|z = 1),$$

where $Pr(s_j = 0|z = 1)$ is the probability of surviving a period j, given that the animal is susceptible. Highly endure animals will have higher probabilities of surviving each sub-period and thus also higher probability of surviving until end of follow-up period. Putative non-susceptible animals will always survive.

For animals that die during the follow up period, susceptibility status is known ($z = 1$), while for surviving animals the true susceptibility status is not observable. Still, for these individuals the probability of being susceptible can be calculated as:

$$Pr(z = 1|c = 0, t) = \frac{Pr(c = 0|t,\ z = 1)Pr(z = 1)}{Pr(c = 0|t)}$$

The proposed cure model allows for individual variation in both prior probability of being susceptible as well as in the endurance of susceptible animals (Ødegård et al., 2011b,c). A detailed description of the cure model is given in Ødegård et al. (2011c).

Box 3 | A normal mixture model.

In a mixed population of infected ($z = 1$) and healthy ($z = 0$) animals, the density of an observation y can be written as:

$$P(y) = P(y|z = 0)Pr(z = 0) + P(y|z = 1)Pr(z = 1).$$

The probability of an animal being infected is thus:

$$Pr(z = 1|y) = \frac{P(y|z = 1)Pr(z = 1)}{P(y|z = 0)Pr(z = 0) + P(y|z = 1)Pr(z = 1)}.$$

A detailed description of the normal mixture model is given in Ødegård et al. (2003, 2005).

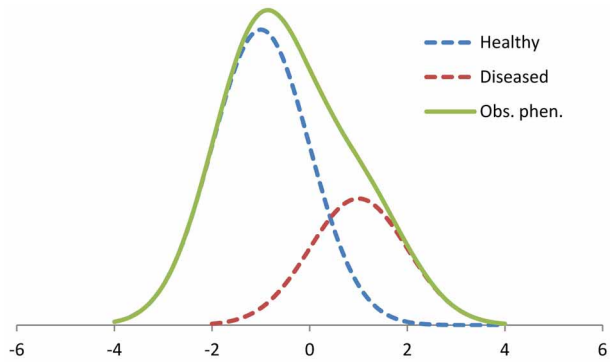

FIGURE 4 | An example of a two-component mixture distribution. The dotted lines are the unobserved distributions of non-infected "healthy" individuals (70% of the observation) with $\sim N(-1.0, 1.0)$ and infected "diseased" individuals (30%) with $\sim N(1.0, 1.0)$. The solid line represents the resulting distribution of the observed phenotypes. Trait values are given on x-axis and the frequencies of observations on y-axis.

selective breeding should focus on susceptibility. This implies that the follow-up period should continue until the vast majority of susceptible animals have died, ensuring that the observed end-survival largely resembles the fraction of non-susceptible animals in the population.

NORMAL MIXTURE MODELS

Normal mixture models can be used to analyze genetics of host performance, e.g., growth rate, within a population consisting of individuals affected and unaffected by a pathogen, even in the absence of pathogen burden and time-until-death data.

Finite normal mixture models have earlier been proposed for analysis of infection-affected, continuously distributed phenotypes, assuming that the true infection statuses of individuals are unknown (Detilleux and Leroy, 2000; Ødegård et al., 2003, 2005; Gianola et al., 2004). The mixture model attempts to identify hidden categories (e.g., non-infected and infected) among the observations, assuming that the continuous scale observations originate from two normal distributions differing in mean and (potentially) variance (**Box 3**; **Figure 4**). For instance, the broiler ascites example given in **Figure 2** can be analyzed using a mixture model analysis assuming that the heart ratio has two underlying distributions, one for non-infected and one for ascitic birds (Zerehdaran et al., 2006). Another example of a mixture trait is somatic cell scores in milk of dairy cattle (Madsen et al., 2008). Somatic cell score is at low level in non-infected cows, but increase to high levels in cases of (unobserved) subclinical mastitis. Hence, the observed somatic cell scores may be viewed as a mixture of two normal distributions (non-infected and mastitic). In Atlantic salmon *Salmo salar* L., diseases such as infectious pancreas necrosis and pancreas disease can kill a fraction of the animals, but may also reduce subsequent growth of affected survivors. Hence, after an outbreak, observed growth of survivors may be viewed as a mixture trait depending on the individuals' previous health status.

Classical selection aims at changing a trait in the desired direction. However, for mixture traits the variation is partly explained by mixing of the two (or more) sub-distributions with different means, and partly by variation within each sub-distribution (**Figure 4**). Hence, if the aim is to reduce the incidence of the infection rather than altering the observed continuous host trait

itself, simple directional selection for the latter (e.g., for somatic cell score) may not be optimal. The mixture model opens new possibilities for selection, and can be used to directly select for reduced infection risk. Additionally, the trait recorded on infected and non-infected animals may be viewed as two distinct sub-traits whose genetic variances and their genetic correlation can be estimated. This resembles the G × E analysis performed with random regression models (**Figure 2**) with the difference that the mixture model does not take into account that infected individuals may have different pathogen burdens.

Normal mixture models typically assume that an individual is either infected or not, and that infection has a certain effect on the phenotype (**Figure 4**). However, variation in environmental pathogen load and in individual tolerance for the infection imply that the effect of an infection may vary substantially among individuals and environments. The proposed mixture models may be extended to allow for individual responses to infection (Madsen et al., 2008). Alternatively, the model may be extended to a growth mixture model (Wang and Bodner, 2007). The growth mixture models assume that the observations come from different latent trajectories, i.e., health status does not only affect the expectation of individual observations, but also the slope of a phenotypic trajectory (growth curves). Infected and non-infected animals could show different trajectories, with the non-infected ones being unaffected by the pathogen, while the infected individuals being variably affected by the pathogen burden. Such models may be useful to analyze resistance and infection-affected traits observed on animals with unknown infection status and in environments with variable pathogen loads.

APPLICATION OF THE METHODS IN BREEDING PROGRAMMES

Random regression models are routinely applied in farm animal breeding programs, e.g., for milk test-day models in dairy cows and for growth curves (Schaeffer, 2004). Similarly, random regression models can be implemented to select for tolerance, given suitable data are available. The cure model approach for the analysis of time-until-death data (Veerkamp et al., 2001; Ødegård et al., 2011a) have been implemented in the DMU software, allowing the estimation of genetic parameters and breeding values for practical breeding (Madsen and Jensen, 2010). To our knowledge, the cure model has not been implemented in routine genetic evaluations in any breeding program. Árnason (1999) and Urioste et al. (2007) have proposed a bivariate linear-threshold model which can be used to analyze whether an animal survived (a threshold trait) and how long it took until death (a linear trait). Such a model resembles the cure model and is straightforward to apply in multi-trait breeding value evaluations. Also the normal mixture model has been implemented in the DMU software (Madsen and Jensen, 2010), and is therefore available for multi-trait genetic evaluations, but to our knowledge, has not yet been implemented in routine genetic evaluations.

The cure model has been applied to survival data in aquaculture species, leading to altered recommendations for routine disease-challenge testing (Ødegård et al., 2011b). Historically, challenge tests in aquaculture species have been terminated at intermediate cumulative mortalities to ensure maximum variation in binary survival data. However, this approach is only proper given that endurance and susceptibility are equivalent traits, which is not necessarily the case. The current advice is to continue testing until mortality naturally ceases, even at levels above 50% mortality (Ødegård et al., 2011a).

So far, only a limited number of breeding programs have considered selecting for tolerance. Some African cattle breeding programs are specifically selecting for trypanotolerance-related traits, the tolerance being a major breeding objective trait (Hanotte et al., 2003; van der Waaij et al., 2003). In contrast, regardless of the extensive studies conducted in Australia and New Zealand on nematode tolerance in sheep, a decision has been made not to record and select for tolerance because of the need to let animals to suffer and production to be reduced for tolerance to be expressed (Albers et al., 1987; Bisset and Morris, 1996; Woolaston and Windon, 2001). The novel statistical methods and the increasing awareness of the detailed physiological mechanisms of tolerance (Medzhitov et al., 2012) may provide more opportunities for tolerance selection in farm animals.

CONCLUSIONS

The recent statistical developments provide tools to increase our understanding of genetics of alternative strategies to defend against parasites, pathogens, and production diseases. Most of the statistical methods can be applied in breeding value evaluations to breed for tolerance. Moreover, the methods presented here provide tools to quantify genotype-by-pathogen burden interactions that may explain a significant proportion of phenotypic variation in traits within populations that are exposed to various infections and production diseases. The traits whose variation is affected are typically production traits that are selected for in breeding programs. To be able to unambiguously select for the genetic potential of a production trait, the effects of resistance and tolerance should be separated from it. The methods presented in this paper provide potential to construct more effective breeding programs to increase both productivity and animal health.

REFERENCES

Albers, G. A., Gray, G. D., Piper, L. R., Barker, J. S., Le Jambre, L. F., and Barger, I. A. (1987). The genetics resistance and resilience to *Haemonchus contortus* infection in young merino sheep. *Int. J. Parasitol.* 17, 1355–1363.

Arendt, J. D. (1997). Adaptive intrinsic growth rates: an integration across taxa. *Q. Rev. Biol.* 72, 149–177.

Árnason, T. (1999). Genetic evaluation of Swedish standard-bred trotters for racing performance traits and racing status. *J. Anim. Breed. Genet.* 116, 387–398.

Best, A., White, A., and Boots, M. (2008). Maintenance of host variation in tolerance to pathogens and parasites. *Proc. Natl. Acad. Sci. U.S.A.* 105, 20786–20791.

Bishop, S. C., and MacKenzie, K. M. (2003). Genetic management strategies for controlling infectious diseases in livestock populations. *Genet. Sel. Evol.* 35, S3–S17.

Bisset, S. A., and Morris, C. A. (1996). Feasibility and implications of breeding sheep for resilience to nematode challenge. *Int. J. Parasitol.* 26, 857–868.

Bloemhof, S., Kause, A., Knol, E. F., van Arendonk, J. A. M., and Misztal, I. (2012). Heat stress effects on farrowing rate in sows: genetic parameter estimation using within-line and crossbred models. *J. Anim. Sci.* 90, 2109–2119.

Calus, M. P. L., Bijma, P., and Veercamp, R. F. (2004). Effects of data structure on the estimation of covariance functions to

describe genotype by environment interactions in a reaction norm model. *Genet. Sel. Evol.* 36, 489–507.

Charmantier, A., and Garant, D. (2005). Environmental quality and evolutionary potential: lessons from wild populations. *Proc. R. Soc. Ser. B* 272, 1415–1425.

Charmantier, A., Kruuk, L. E., and Lambrechts, M. M. (2004). Parasitism reduces the potential for evolution in a wild bird population. *Evolution* 58, 203–206.

Clunies-Ross, I. (1932). Observations on the resistance of sheep to infestations by the stomach worm *Haemonchus contortus*. *J. Coun. Sci. Ind. Res.* 5, 73–80.

de Greef, K. H., Janss, L. L., Vereijken, A. L., Pit, R., and Gerritsen, C. L. (2001). Disease-induced variability of genetic correlations: ascites in broilers as a case study. *J. Anim. Sci.* 79, 1723–1733.

Detilleux, J., and Leroy, P. L. (2000). Application of a mixed normal mixture model for the estimation of mastitis-related parameters. *J. Dairy Sci.* 83, 2341–2349.

Ducrocq, V., and Casella, G. (1996). A Bayesian analysis of mixed survival models. *Genet. Sel. Evol.* 28, 505–529.

Falconer, D. S. (1952). The problem of environment and selection. *Am. Nat.* 86, 293–298.

Farewell, V. T. (1982). The use of mixture-models for the analysis of survival-data with long-term survivors. *Biometrics* 38, 1041–1046.

Gianola, D., Ødegård, J., Heringstad, B., Klemetsdal, G., Sorensen, D., Madsen, P., et al. (2004). Mixture model for inferring susceptibility to mastitis in dairy cattle: a procedure for likelihood-based inference. *Genet. Sel. Evol.* 36, 3–27.

Hanotte, O., Ronin, Y., Agaba, M., Nilsson, P., Gelhaus, A., Horstmann, R., et al. (2003). Mapping of quantitative trait loci controlling trypanotolerance in a cross of tolerant West African N'Dama and susceptible East African Boran cattle. *Proc. Natl. Acad. Sci. U.S.A.* 100, 7443–7448.

Henderson, C. R. (1982). Analysis of covariance in the mixed model: higher-level, non homogeneous, and random regressions. *Biometrics* 38, 623–640.

Hoffmann, A. A., and Merilä, J. (1999). Heritable variation and evolution under favourable and unfavourable conditions. *Trends Ecol. Evol.* 14, 96–101.

Kause, A. (2011). Genetic analysis of tolerance to infections using random regressions: a simulation study. *Genet. Res.* 93, 291–302.

Kause, A., and Morin, J. P. (2001). Seasonality and genetic architecture of development time and body size in the birch feeding sawfly *Priophorus pallipes*. *Genet. Res.* 78, 31–40.

Kause, A., Ritola, O., and Paananen, T. (2007). Changes in the expression of genetic characteristics across cohorts in skeletal deformations of farmed salmonids. *Genet. Sel. Evol.* 39, 529–543.

Kause, A., Ritola, O., Paananen, T., Wahlroos, H., and Mäntysaari, E. A. (2005). Genetic trends in growth, sexual maturity and skeletal deformations, and rate of inbreeding in a breeding programme for rainbow trout. *Aquaculture* 247, 177–187.

Kause, A., van Dalen, S., and Bovenhuis, H. (2012). Genetics of ascites resistance and tolerance in chicken: a random regression approach. *G3* 2, 527–535.

Kirkpatrick, M., Lofsvold, D., and Bulmer, M. (1990). Analysis of the inheritance, selection and evolution of growth trajectories. *Genetics* 124, 979–993.

Kolmodin, R., and Bijma, P. (2004). Response to mass selection when the genotype by environment interaction is modelled as a linear reaction norm. *Genet. Sel. Evol.* 36, 435–454.

Kuukka-Anttila, H., Peuhkuri, N., Kolari, I., Paananen, T., and Kause, A. (2010). Quantitative genetic architecture of parasite-induced cataract in rainbow trout, *Oncorhynchus mykiss*. *Heredity* 104, 20–27.

Leimu, R., and Koricheva, J. (2006). A meta-analysis of trade-offs between plant tolerance and resistance to herbivores: combining the evidence from ecological and agricultural studies. *Oikos* 112, 1–9.

Lewis, C. R. G., Torremorell, M., Galina-Pantoja, L., and Bishop, S. C. (2009). Genetic parameters for performance traits in commercial sows estimated before and after an outbreak of porcine reproductive and respiratory syndrome. *J. Anim. Sci.* 87, 876–884.

Lillehammer, M., Ødegård, J., and Meuwissen, T. H. E. (2009). Reducing the bias of estimates of genotype-by-environment interactions in random regression sire models. *Genet. Sel. Evol.* 41, 30.

Madsen, P., and Jensen, J. (2010). DMU: A user's guide. A package for analysing multivariate mixed models. Version 6, release 5.0. Available Online at: http://dmu.agrsci.dk/dmuv6_guide.5.0.pdf

Madsen, P., Shariati, M. M., and Ødegård, J. (2008). Genetic analysis of somatic cell score in Danish Holsteins using a liability-normal mixture model. *J. Dairy Sci.* 91, 4355–4364.

Mauricio, R., Rausher, M. D., and Burdick, D. S. (1997). Variation in the defense strategies of plants: are resistance and tolerance mutually exclusive? *Ecology* 78, 1301–1311.

Medzhitov, R., Schneider, D. S., and Soares, M. P. (2012). Disease tolerance as a defense strategy. *Science* 6071, 936–941.

Meyer, K., and Hill, W. G. (1997). Estimation of genetic and phenotypic covariance functions for longitudinal or 'repeated' records by restricted maximum likelihood. *Livest. Prod. Sci.* 47, 185–200.

Ødegård, J., Baranski, M., Gjerde, B., and Gjedrem, T. (2011a). Methodology for genetic evaluation of disease resistance in aquaculture species: challenges and future prospects. *Aquacult. Res.* 42, 103–114.

Ødegård, J., Gitterle, T., Madsen, P., Meuwissen, T. H. E., Yazdi, H. Y., Gjerde, B., et al. (2011b). Quantitative genetics of taura syndrome resistance in pacific white shrimp (*Penaeus vannamei*): a cure model approach. *Genet. Sel. Evol.* 43, 14.

Ødegård, J., Madsen, P., Labouriau, R., Gjerde, B., and Meuwissen, T. H. E. (2011c). A sequential threshold cure model for genetic analysis of time-to-event data. *J. Anim. Sci.* 89, 943–950.

Ødegård, J., Jensen, J., Madsen, P., Gianola, D., Klemetsdal, G., and Herinstad, B. (2003). Detection of mastitis in dairy cattle by use of mixture models for repeated somatic cell scores: a Bayesian approach via Gibbs sampling. *J. Dairy Sci.* 86, 3694–3703.

Ødegård, J., Madsen, P., Gianola, D., Klemetsdal, G., Jensen, J., Heringstad, B., et al. (2005). A Bayesian threshold-normal mixture model for analysis of a continuous mastitis-related trait. *J. Dairy Sci.* 88, 2652–2659.

Painter, R. H. (1958). Resistance of plants to insects. *Annu. Rev. Entomol.* 3, 267–290.

Pakdel, A., van Arendonk, J. A. M., Vereijken, A. L., and Bovenhuis, H. (2005). Genetic parameters of ascites-related traits in broilers: effect of cold and normal temperature conditions. *Br. Poult. Sci.* 46, 35–42.

Rausher, M. D. (2001). Co-evolution and plant resistance to natural enemies. *Nature* 411, 857–864.

Rauw, W. M., Kanis, E., Noordhuizen-Stassen, E. N., and Grommers, F. J. (1998). Undesirable side effects of selection for high production efficiency in farm animals: a review. *Livest. Prod. Sci.* 56, 15–33.

Ravagnolo, O., and Misztal, I. (2000a). Genetic component of heat stress in dairy cattle: parameter estimation. *J. Dairy Sci.* 83, 2126–2130.

Ravagnolo, O., and Misztal, I. (2000b). Effect of heat stress on nonreturn rate in Holstein cows: genetic analyses. *J. Dairy Sci.* 85, 3092–3100.

Schaeffer, L. R. (2004). Application of random regression models in animal breeding. *Livest. Prod. Sci.* 86, 35–45.

Schat, H., Llugany, M., Vooijs, R., Hartley-Whitaker, J., and Bleeker, P. M. (2002). The role of phytochelatins in constitutive and adaptive heavy metal tolerances in hyperaccumulator and non-hyperaccumulator metallophytes. *J. Exp. Bot.* 53, 2381–2392.

Serenius, T., and Stalder, K. J. (2004). Genetics of length of productive life and lifetime prolificacy in the Finnish Landrace and Large White pig populations. *J. Anim. Sci.* 82, 3111–3117.

Simms, E. L. (2000). Defining tolerance as a norm of reaction. *Evol. Ecol.* 14, 563–570.

Simms, E. L., and Triplett, J. (1994). Costs and benefits of plant responses to disease: resistance and tolerance. *Evolution* 48, 1973–1985.

Stear, M. J., Bairden, K., Duncan, J. L., Gettinby, G., McKellar, Q. A., Murray, M., et al. (1995). The distribution of faecal nematode egg counts in Scottish Blackface lambs following natural, predominantly *Ostertagia circumcincta* infection. *Parasitology* 110, 573–581.

Tiffin, P., and Inouye, B. D. (2000). Measuring tolerance to herbivory: accuracy and precision of estimates made using natural versus imposed damage. *Evolution* 54, 1024–1029.

Urioste, J. I., Misztal, I., and Bertrand, J. K. (2007). Fertility traits in spring-calving Aberdeen Angus cattle. 1. Model development and genetic parameters. *J. Anim. Sci.* 85, 2854–2860.

van der Waaij, E., Hanotte, O., van Arendonk, J. A. M., Kemp, S. J., Kennedy, D., Gibson, A., et al. (2003). Population parameters for traits defining trypanotolerance in

an F2 cross of N'Dama and Boran cattle. *Livest. Prod. Sci.* 84, 219–230.
van der Waaij, E. H., Bijma, P., Bishop, S. C., and van Arendonk, J. A. M. (2000). Modeling selection for production traits under constant infection pressure. *J. Anim. Sci.* 78, 2809–2820.
Veerkamp, R. F., Brotherstone, S., Engel, B., and Meuwissen, T. H. E. (2001). Analysis of censored survival data using random regression models. *Anim. Sci.* 72, 1–10.
Vehviläinen, H., Kause, A., Quinton, C., Koskinen, H., and Paananen, T. (2008). Survival of the currently fittest – Genetics of rainbow trout survival across time and space. *Genetics* 180, 507–516.
Vehviläinen, H., Kause, A., Quinton, C., Koskinen, H., and Paananen, T. (2010). Genetic architecture of rainbow trout survival from egg to adult. *Genet. Res.* 92, 1–11.
Via, S., and Lande, R. (1985). Genotype-environment interaction and the evolution of phenotypic plasticity. *Evolution* 39, 505–522.
Wang, M., and Bodner, T. E. (2007). Growth mixture modeling. *Organ. Res. Methods* 10, 635–656.
Wideman, R. F. Jr., Wing, T., Kirby, Y. K., Forman, M. F., Marson, N., Tackett, C. D., et al. (1998). Evaluation of minimally invasive indices for predicting ascites susceptibility in three successive hatches of broilers exposed to cool temperatures. *Poult. Sci.* 77, 1565–1573.
Windig, J. J., Mulder, H. A., Bohthe-Wilhelmus, D. I., and Veerkamp, R. F. (2011). Simultaneous estimation of genotype-by-environment interaction accounting for discrete and continuous environmental descriptors in Irish dairy cattle. *J. Dairy Sci.* 94, 3137–3147.
Woolaston, R. R., and Windon, R. G. (2001). Selection of sheep for response to *Trichostrongylus colubriformis* larvae: genetic parameters. *Anim. Sci.* 73, 41–48.
Zerehdaran, S., van Grevehof, E. M., van der Waaij, E. H., and Bovenhuis, H. (2006). A bivariate mixture model analysis of body weight and ascites traits in broilers. *Poult. Sci.* 85, 32–38.

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The molecular pathways underlying host resistance and tolerance to pathogens

Elizabeth J. Glass*

The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, UK

Edited by:
Ilias Kyriazakis, Newcastle University, UK

Reviewed by:
Ikhide G. Imumorin, Cornell University, USA
David Kerr, University of Vermont, USA

****Correspondence:***
Elizabeth J. Glass, The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush, Midlothian, EH25 9RG, UK.
e-mail: liz.glass@roslin.ed.ac.uk

Breeding livestock that are better able to withstand the onslaught of endemic- and exotic pathogens is high on the wish list of breeders and farmers world-wide. However, the defense systems in both pathogens and their hosts are complex and the degree of genetic variation in resistance and tolerance will depend on the trade-offs that they impose on host fitness as well as their life-histories. The genes and pathways underpinning resistance and tolerance traits may be distinct or intertwined as the outcome of any infection is a result of a balance between collateral damage of host tissues and control of the invading pathogen. Genes and molecular pathways associated with resistance are mainly expressed in the mucosal tract and the innate immune system and control the very early events following pathogen invasion. Resistance genes encode receptors involved in uptake of pathogens, as well as pattern recognition receptors (PRR) such as the toll-like receptor family as well as molecules involved in strong and rapid inflammatory responses which lead to rapid pathogen clearance, yet do not lead to immunopathology. In contrast tolerance genes and pathways play a role in reducing immunopathology or enhancing the host's ability to protect against pathogen associated toxins. Candidate tolerance genes may include cytosolic PRRs and unidentified sensors of pathogen growth, perturbation of host metabolism and intrinsic danger or damage associated molecules. In addition, genes controlling regulatory pathways, tissue repair and resolution are also tolerance candidates. The identities of distinct genetic loci for resistance and tolerance to infectious pathogens in livestock species remain to be determined. A better understanding of the mechanisms involved and phenotypes associated with resistance and tolerance should ultimately help to improve livestock health and welfare.

Keywords: genetics, breeding, disease resistance, tolerance, livestock, immunity, inflammation, pathogen

INTRODUCTION

Selective breeding strategies for livestock species have been employed to great advantage for the human race, creating new breeds with improved productivity traits such as increased milk yield and faster growth. This process has gained momentum in recent decades with advances in technologies and resources to achieve more targeted breeding. Thus, the process of selection in species of agricultural importance has changed from relying on readily observable phenotypes e.g., coat color, to employing high density SNP chips and genomic prediction of specific production traits (reviewed by Hume et al., 2011). We are now experiencing a genomics information explosion with the advent of cheaper and faster sequencing of genomes. Aims currently include sequencing multiple individuals within species including a project to sequence 1000 cattle genomes (www.1000bullgenomes.com). In theory, this type of information could provide sufficient knowledge and resources for genetic variation that could ever be needed to target selective breeding for specific traits. Furthermore, strong evidence has accumulated that livestock species from birds to mammals, harbor genes that control protective responses to the various classes of pathogen from viruses to complex metazoans such as nematodes (for reviews that comprehensively cover different livestock species and pathogens see e.g., Davies et al., 2009; Mirkena et al., 2010). However, there remains a "phenotype gap" for traits linked to disease resistance and tolerance (Glass et al., 2012a,b). This is partly because host-pathogen interactions are highly complex, involving many different molecules and cell types which interact together over time. Invoking the wide arsenal of defense mechanisms in the host is partly dependent on the pathogen and partly on other factors such as the physiological state of the animal as well as previous exposure. Thus, the outcome—protection and survival or disease and potentially death—and the relationship of these to a measurable outcome of fitness, which in the case of livestock is usually equated to traits such as growth or yield, is difficult to predict. Furthermore, the correlates of protection or pathogenesis are often unclear and even under more straightforward situations, the logistical difficulties of measuring relevant phenotypes at the most appropriate time points in livestock in the field can be formidable. In addition, many factors, both genetic and non-genetic influence the outcome of exposure to pathogens. Nowhere is this more of an issue than considerations of what parameters to measure in order to ascertain whether an animal is resistant or tolerant to a pathogen. This article will explore from an immunologist's point

of view, the definitions of "resistance" and "tolerance," how to measure them, and whether they are separately determined traits controlled by many non-overlapping genes. The main focus is on identifying the genes and molecular pathways that underpin host defense from pathogens, and are likely candidates for resistance and tolerance traits.

RESISTANCE AND TOLERANCE: DEFINITION AND FITNESS TRADE-OFFS

In the context of this review and special issue, resistance is defined as an ability to reduce pathogen replication in the host, whereas tolerance is defined as an ability to maintain homeostasis in the presence of a replicating pathogen, with limited ensuing pathology (see Doeschl-Wilson et al., 2012a,b). Thus, resistance traits may be considered as those governed by genes that function as barriers to pathogen entry as well as genes expressed during an active innate or acquired immune response to the pathogen that result in a reduction of pathogen burden. In contrast, tolerance traits may be controlled by genes that suppress or otherwise limit active responses to the pathogen and/or genes that prevent pathogen mediated toxicity, but have no effect on pathogen burden. Indeed, many diseases are caused by collateral damage of the host's own tissues during the process of immune-related defense mechanisms, i.e., immunopathology, rather than toxicity caused by the pathogen itself. For example, young cattle infected with bovine respiratory syncytial virus (BRSV), show considerable lung pathology that appears to be linked to an influx of immune cells, whereas infection of bovine epithelial cells *in vitro* with BRSV do not show cytopathology (Valarcher and Taylor, 2007). Although there is considerable literature to suggest that, there is a genetic component to the response to BRSV (Glass et al., 2010, 2012a; Leach et al., 2012), whether this relates to resistance and/or tolerance is unclear. Additionally, an ability to protect the host from damage caused by pathogen derived toxicity could also be a component of a tolerance phenotype (Medzhitov, 2009).

Thus, for a host species to survive there needs to be a balance between protection against the onslaught of infection, and the consequences of immunopathology and direct toxicity by the pathogen. The selective pressure exerted by pathogens on their hosts drives the evolution of counter-measures and vice versa (Woolhouse et al., 2002). This co-evolution may result in the development of resistance or tolerance mechanisms in the host (Carval and Ferriere, 2010), and virulence factors (Ebert and Bull, 2003) or ways of evading or subverting the host immune defense mechanisms in the pathogen (Schmid-Hempel, 2009). These host-pathogen interactions across time leave their mark on the host genome in terms of polymorphisms in genes underpinning resistance and tolerance traits. However, the complexity of these interactions together with heterogeneous environmental factors makes it difficult to predict optima or outcomes (Lazzaro and Little, 2009).

The evidence that different genes control disease resistance and tolerance was originally obtained from plant studies, which demonstrated that genetic variation in both resistance and tolerance existed (Simms and Triplett, 1995). Gene variants that confer greater resistance to pathogens are predicted to be unlikely to go to fixation in a population, because although they effectively reduce the levels of pathogen burden, their fitness costs outweigh the costs of retaining the resistance traits in the absence of infection (Roy and Kirchner, 2000). In contrast, if a host evolves more effective tolerance mechanisms, it has been hypothesized that these would no longer act as further selective pressure on the pathogen (Roy and Kirchner, 2000). Increased frequencies of tolerant individuals would lead to a rise in pathogen burden in a population, and thus, any fitness benefits of tolerance are predicted to drive tolerance traits to fixation (Roy and Kirchner, 2000). The eventual outcome in such cases might achieve an equilibrium in which host populations become completely tolerant to the surrounding pathogens. These may then be observed as endemic or even as commensals or environmental micro-organisms (Medzhitov, 2009; Nussbaum and Locksley, 2012).

However, these conclusions make assumptions that resistance always confers a negative fitness cost and that tolerance always confers a positive cost, whereas in plant studies, the estimated costs of resistance and tolerance do not necessarily follow evolutionary theory in that fitness is not necessarily compromised by resistance to pathogens, nor is tolerance necessarily beneficial to fitness (Simms and Triplett, 1995). Thus, it has been argued that the relationship between resistance and tolerance is dependent on the trade-offs each impose on the host in terms of fitness (Restif and Koella, 2004; Carval and Ferriere, 2010).

The trade-offs also depend on the virulence of the infecting organism, which raises another consideration: virulence of the infecting organism is intimately associated with the response by its host (Margolis and Levin, 2008). The definition of virulence is still widely debated in the literature, with the debate ranging from the micro-organism perspective to the host. Many define virulence as the ability of a micro-organism to multiply in a host and cause harm (Poulin and Combes, 1999) i.e., the capacity to infect and ability to transmit, which relates to pathogen fitness (Kirchner and Roy, 2002). However, virulence in relation to animals is commonly defined as a pathogen-induced reduction in host fitness, which is dependent on pathogen dose and is therefore, a consequence of host-pathogen interactions (Casadevall and Pirofski, 2001; Margolis and Levin, 2008). This is the definition used in this review. Virulence is usually measured by the level of host mortality, but often for practical and ethical reasons the degree of host morbidity is used instead (Alizon et al., 2009). However, virulence can be attributed to both intrinsic virulence factors of the micro-organism, which can cause direct toxicity as well as damage caused by the host response to the micro-organism (Day et al., 2007; Best et al., 2012). Furthermore, what is harmful to one host species may exist as a commensal in another species (Casadevall and Pirofski, 2001). Thus, for example, *Escherichia coli 0157* or *Salmonella spp* are carried and shed by livestock with little apparent ill effect on these host species (Stevens et al., 2009; Clermont et al., 2011). However, these micro-organisms can cause serious consequences in humans and indeed in young or old livestock. This highlights the fact that the *same* micro-organism can have very different effects-dependent on the host species and its physiological state. Further the consequences of infection are also determined by the micro-organism's

route of entry or translocation from one host niche to another. For example, many commensal gut bacteria only cause disease when the gut epithelia is compromised (Pamer, 2007) or the human nasopharynx commensal, *Neisseria meningitides*, only causes severe meningitis or septicaemia if it is able to invade other tissue compartments (Trivedi et al., 2011).

Moreover, another reason that resistance or tolerance genes might not go to fixation in populations may be because the gene variants predispose carriers to infection with a different pathogen (Dean et al., 2002). Indeed, Ayres and Schneider (2008) found that when they infected a mutant Drosophila line, with a range of different bacteria, the single mutation had effects on both resistance and tolerance, the degrees of which were dependent on the pathogen. Similar findings have been reported by Marsh et al. (2011) in which they showed that the nematode, *Caenorhabditis elegans* which lacked a single lysozyme gene, *LYS-7*, had diminished resistance to *Cryptococcus neoformans*, and other pathogens, but enhanced tolerance to *Salmonella typhimurium*.

Thus, in contrast to the prevailing view that host tolerance genes as opposed to host resistance genes, will inevitably evolve to fixation, it seems more likely that the complexity of host-pathogen interactions will inevitably led to observed variation in both tolerance and resistance traits.

More recently evidence for underlying genetic differences in tolerance and resistance traits has been gleaned from a few experimental animal models—mainly from invertebrates such as Drosophila (Corby-Harris et al., 2007; Ayres and Schneider, 2008, 2009), butterflies (Lefevre et al., 2011), or Daphnia (Graham et al., 2011). However, a study by Raberg et al. (2007) indicated that different strains of mice differed in their tolerance to a *Plasmodium* parasite. Weight loss and anemia were shown to correlate with morbidity or fitness, and the authors used these to identify reaction norms of the slope of host fitness against parasite burden following challenge, in individuals from five genetically distinct strains of mice. They found that, there were significant differences in the reaction norms between mouse strains. This implies that mammals also harbor gene variants that control tolerance traits as well as resistance traits. In addition, they found that the mouse strain that was the most tolerant was the least resistant in terms of peak parasite burden, and *vice versa*, suggesting that reduced tolerance is a cost of resistance—at least in this example. A more recent study has extended these principles to wild fish (Blanchet et al., 2010). However, no genes or mechanisms underpinning these traits have been identified in either of these latter studies. Taken together, all of these studies also suggest that different genes can control disease resistance and tolerance, and that they can be antagonistic. Nonetheless other models suggest that this relationship is not inevitable and resistance and tolerance traits can be independent (Simms and Triplett, 1995; Restif and Koella, 2004). Ayres and Schneider (2008) suggest it is likely that both tolerance and resistance mechanisms need to be evoked to ensure survival, and they propose that a variety of different scenarios could be envisaged for each mechanism ranging from high resistance/low tolerance and *vice versa*, and any state in between.

Thus, genetic variants for resistance and tolerance in populations are likely to depend on previous histories of exposure to pathogens, the types of pathogen and the trade-offs they impose on host fitness. It should therefore be possible to select for resistance, tolerance, or potentially for both traits together in livestock populations, but importantly, the goal will depend on the characteristics of the pathogen and what effect it has on the host. However, the studies described above also indicate that achieving optimum resistance or tolerance to a range of pathogens might prove difficult.

GENETICS OF RESISTANCE AND TOLERANCE IN LIVESTOCK: WHAT NEEDS TO BE MEASURED?

Identifying the underlying genes for resistance and tolerance in livestock is likely to be difficult, especially in the case of tolerance traits. Nonetheless, evidence that animals also exhibit genetic variation in resistance and tolerance (Raberg et al., 2007, 2009; Blanchet et al., 2010) would suggest that livestock may also harbor selectable gene variants for these traits.

Most studies on genes and their variants related to host responses to pathogens essentially refer to them as disease resistance genes or loci or traits. However, some of these may more correctly be related to tolerance. The problem is that resistance and tolerance are not clearly distinguished and often do not measure appropriate parameters of "fitness" such as growth, weight, health, or reproductive success. Although sheep and cattle breeds and individuals have been described as having variable tolerance to several infections by pathogens e.g., nematodes (Mirkena et al., 2010), and trypanosomes (Noyes et al., 2011), the term tolerance is not well-defined and generally appears to relate to resilience rather than to a demonstration that the tolerant animals' performance and reproductive traits are maintained in the absence of infection, and/or regardless of pathogen burden to a greater degree than non-tolerant animals. This is not to say that, these are not descriptions of tolerance to infection, simply, the relevant parameters have often not been measured. In addition, as argued elsewhere in this special edition (Doeschl-Wilson et al., 2012b), defining tolerance based on groups of individuals makes the estimation of the effect of tolerance traits less accurate, especially for outbred species such as livestock.

In order to distinguish between resistance and tolerance as defense strategies additional data collection is required as pathogen burden also has to be measured, yet it is clear that pathogen burden does not necessarily have a linear relationship with either resistance or tolerance (Viney et al., 2005; Stjernman et al., 2008; Graham et al., 2011). Thus, for example Stjernman et al. (2008) found that, when the fitness cost of host resistance to a parasite is high, then at *both* low and high parasite burden, host fitness costs may be less than at an intermediate parasite burden. Additionally health or performance traits should be measured both pre-infection as well as post-infection as a measure of constitutive health (Graham et al., 2011; Doeschl-Wilson et al., 2012b). This poses further logistical issues as it is not necessarily clear what samples might need to be collected, from which tissues, or at what time following infection. Many pathogens are not easy to detect e.g., *Mycobacteria* spp., and diagnostic tests can be complex with less than optimal specificities and sensitivities (Wadhwa et al., 2012). Nonetheless in order to begin to understand how host genetic variation impacts on resistance and tolerance traits,

these relationships between pathogen burden and fitness must be assessed.

In addition to obtaining appropriate data on pathogen burden and its relation to host fitness, in order to ascertain the mechanisms that underlie resistance (or tolerance) in livestock, other parameters of the host response to the pathogen need to be measured. This is not straightforward as often the correlates of protection are not clear, therefore, what the most appropriate parameters to measure can difficult to determine. Immune defenses are highly complex, and the types of protective responses differ between pathogens, making it unlikely that a single parameter will be sufficient to determine the underlying molecular pathways. Furthermore, it is easier to measure some parameters than others e.g., systemic antibody responses can be monitored from small stored serum samples, unlike localized cellular responses in large numbers of animals. Yet for many infections, protective mechanisms involve diverse immune responses that also differ across time (Glass et al., 2012a,b; Leach et al., 2012). Additionally, the relationship between immune measures and protection or indeed, "fitness" is usually not clear (Graham et al., 2010; Schneider, 2011). Cost and logistics generally preclude specific challenge studies using carefully calibrated doses. In large-scale studies, using field data, there are often limited information e.g., only veterinary observations, which reduce the accuracy of any estimates of effect. Furthermore, as pointed out by Graham et al. (2011), simply equating stronger immune responses with greater resistance or tolerance can lead to wrong or even diametrically opposed conclusions. In summary, greater consideration of what to measure is necessary to clearly distinguish resistance from tolerance phenotypes.

IDENTITY OF CANDIDATE RESISTANCE AND TOLERANCE GENES

Disease resistance genes are likely to be functional at early stages of pathogen invasion, before it can reach a certain threshold level that would result in host damage. Genetic variants that confer greater resistance should either reduce the pathogen's chances of successful infection or increase the host's rate of pathogen clearance. In contrast, disease tolerance genes should reduce the levels of immunopathology or should enhance the host's ability to protect against pathogen associated toxins. It should be pointed out that even in plants where studies on resistance and tolerance have been undertaken for decades, no specific tolerance genes have been identified to date (Carval and Ferriere, 2010). Although it is now clear that variation in relative resistance and tolerance traits in individuals and strains exist in animal species, for the main part polymorphisms that underpin these traits have not been identified. However, it would seem likely that components of host defense against micro-organisms are likely to play a role. In the next sections a consideration of what molecules and pathways the host employs to defend itself against infection and how they may underpin resistance and tolerance traits. Potential pathogens must first cross the interfaces between the host and its environment. These interfaces consist of the skin and cells lining the mucosal surfaces of the gut, respiratory tract, mammary gland, and genital tract.

HOST RECEPTORS

In order to gain access to the host environment, a pathogen generally does so by binding to host cell surface molecules. One example, where variation in the genes encoding receptors results in host resistance is the C-C chemokine receptor type 5 (CCR5) gene, which is associated with resistance to human immunodeficiency virus (HIV) (Reynes et al., 2001). CCR5 is a chemokine receptor expressed on certain immune cells, but also acts as a receptor for the HIV virus to enter cells. Individuals with a deletion mutation express lower levels of the receptor, which results in lower levels of viral entry, and also in less risk of progression (Reynes et al., 2001). Generally, these individuals are healthy suggesting that this receptor is not essential, but some studies have suggested that lack of it may have a detrimental effect on responses to other pathogens (Dean et al., 2002). Unfortunately, CCR5 is not the only receptor for HIV entry. However, as this mutation reduces the initial infectious dose of HIV, thus lowering the risk of infection and progression, it can be considered as a canonical "resistance" gene.

Many pathogens gain entry via host receptors, but in the case of livestock the majority are unknown. An exception is the gut receptor for *E. coli* F18 encoded by the *fut1* gene in pigs, which confers complete resistance to *E. coli* (Meijerink et al., 1997). In cattle it is known that foot and mouth disease virus (FMDV) enters cells by attaching to the bovine cell surface integrin, $\alpha\upsilon\beta6$ (Monaghan et al., 2005), but whether there are variants that confer resistance is not known. The bovine viral diarrhea virus (BVDV) employs the bovine CD46 cell surface molecule, a member of the complement regulatory receptors, to gain entry to cells (Maurer et al., 2004). Recently, genetic variants in bovine CD46 have been shown to influence cell permissiveness for BVDV, at least *in vitro* (Zezafoun et al., 2011), and thus carriers of CD46 alleles might vary in resistance to BVDV.

HOST DETECTION OF PATHOGENS AND DANGER

Once a pathogen has breached the initial barriers to its entry, the host has a small window of opportunity to detect its presence before it begins to replicate. Therefore, it is of no surprise that the skin and mucosal tracts contain both non-immune cells such as fibroblasts, epithelial and endothelial cells, as well as many innate immune cells which all act as sensors of pathogens and which are highly effective at signaling alarm to the rest of the immune system (Zarember and Godowski, 2002; Matzinger, 2007). Innate defense mechanisms exist in all metazoan species and represent ancient evolutionary protection strategies that probably, first developed in the last common ancestor between animals and plants, even though plants do not contain specialized immune cells (Ronald and Beutler, 2010). These detection systems function to discriminate self from non-self [as originally proposed by Janeway (1989) whose farsighted view was to hypothesize the existence of pattern recognition receptors (PRRs)] and/or to discriminate between agents of potential damage from those which are benign [the "danger" theory as proposed by Matzinger (1994)]. The consensus view, currently, is that both pathogen and non-pathogen associated damage or danger signals may in fact be necessary for initiation of responses to pathogens and that the context in which pathogens are detected is critical for the ensuing

innate immune response (Fontana and Vance, 2011). Since both types of signal are crucial determinants of the strength and nature of the ensuing response to inflammatory signals, they need to be considered as elements of both resistance and tolerance.

Among the earliest detection, systems are pattern or pathogen recognition receptors (PRRs) and molecules (PRMs), which are expressed in most cell types, but importantly in the cells of mucosal surfaces such as epithelial cells and fibroblasts, as well as immune cells such as the antigen presenting cells (APCs), macrophages, and dendritic cells. These receptors recognize conserved molecular structures on pathogens that are not found in hosts, pathogen associated molecular patterns (PAMPs), for example, bacterial cell wall components and double stranded RNA (Kumar et al., 2011). PRRs are present as soluble molecules as well as on cell surfaces and within the cytoplasm and thus can detect both extra- and intra-cellular pathogens (Kersse et al., 2011). An example of an early detection system involving soluble proteins are the trypanolytic factors present only in human and some primate serum, which renders them resistant to most trypanosome species (Vanhollebeke and Pays, 2010). These factors involve apolipoprotein L-1 (APOL-1), which is related to the family of pro and anti-apoptotic Bcl2 molecules and evolved in early primates from a gene duplication event, and an acute phase protein, the soluble PRR, haptoglobin-related protein (Hpr). Sera from other host species such as Cape buffalo also contain factors which kill trypanosomes, but the so-called trypanotolerance trait present in certain breeds of African cattle involves other components of the innate immune system (Namangala, 2012).

PRRs consist of various domains with a variety of functions including ligand recognition, which are highly evolutionarily conserved (Ronald and Beutler, 2010; Hansen et al., 2011). The best known examples of PRR are the family of toll-like receptors (TLR), and subtle differences in sequence across species and within species have been associated with differences in response to a variety of pathogens in many species including livestock (Werling et al., 2009). Indeed we have suggested that they may be the best candidates for selection of animals with lower risk of infections (Jann et al., 2009). In addition, we (and others) have identified signals of positive selection in bovine TLRs (Jann et al., 2008; Smith et al., 2012). However, many other PRRs exist including the C-type lectin receptors, as well as sets of receptors which are important for detecting the presence of cytosolic PAMPs such as RNA and DNA including NOD-like receptors (NLRs), RIG-I-like receptors (RLRs), and DNA receptors (cytosolic sensors for DNA). There are likely more to be discovered and the degree to which they all interact (after all pathogens have many different PAMPs) is still a hot topic of research (Kawai and Akira, 2011).

It also appears that the host is primed to respond to intrinsic inflammatory signals, sometimes referred to as danger or damage signals (Matzinger, 2002; Bianchi, 2007; Lotze et al., 2007). These are essentially host components released or expressed immediately following stress and damage of cells and tissues, and which evoke an inflammatory response. The nature of these intrinsic alarm molecules, sometimes referred to as "alarmins" and their receptors is still not well-understood and their role in pathogen or trauma-induced inflammation remains controversial (Manson et al., 2012).

The term damage-associated molecular patterns (DAMPs) thus can encompass both PAMPs and alarmins (Bianchi, 2007). The receptor candidates for alarmins include PRRs themselves (Seong and Matzinger, 2004), the Receptor for Advanced Glycation End products, RAGE, (Bianchi, 2007), and an APC receptor, CLEC9, recently discovered to recognize actin filaments released by necrotic cells (Ahrens et al., 2012).

HOST INNATE RESPONSES

Such a two signal model would help explain, why hosts respond to pathogenic micro-organisms but not commensals, which also express PAMPs (Nussbaum and Locksley, 2012). In a recent review, Blander and Sander (2012) suggest that recognition and response are likely to involve a whole series of interacting components, and the type and magnitude of response depends on at least five checkpoints. These enable the host to assess the threat of the micro-organisms to host integrity and respond accordingly. First, as micro-organisms have multiple PAMPs, the host initially integrates these signals, resulting in a cascade of intracellular signaling through various adapter molecules (e.g., MyD88), followed by activation of the MAP kinase family, which in turn switches on transcription factors such as NF-kB and interferon regulatory factor (IRF) family members. These down-stream signaling pathways, which are conserved across species, result in the up-regulation of molecules associated with inflammation such as cytokines, and/or induction of autophagy and various cell death pathways leading to synergistic production of cytokines (Bianchi, 2007; Duprez et al., 2009; Hansen et al., 2011). If the host's innate responses are strong and rapid, there is evidence that micro-organisms are cleared and little pathology will be evident (Evans et al., 2010). Any cell death will be well controlled through apoptotic mechanisms, which ensure that the cellular contents are not released to the external milieu. The resulting apoptotic bodies are then engulfed by phagocytic cells such as macrophages (Mφ) (Duprez et al., 2009). Thus, genes controlling these first few hours of response to pathogen invasion are likely gene candidates for resistance traits. However, if this does not result in elimination or destruction of the micro-organism, then the tissue load (Willer et al., 2012), and whether the micro-organism is alive or dead (Fontana and Vance, 2011), in other words sensing micro-organism growth in tissues, may become more important determinants of immunopathology. In this second phase, the host may detect micro-organism derived metabolic molecules such as mRNA or bacterial pyrophosphates. The third checkpoint may be the sensing of virulence factors or their activity, although it has to be said that commensals can also possess virulence factors such as type III secretion systems, and what may count more is whether micro-organisms have breached the mucosal layer (Swiatczak et al., 2011). Possibly hosts actually sense changes in their own metabolism as well, for example changes in transcription and translation (Kleino and Silverman, 2012). One key component appears to be the up-regulation of a transcription factor, ZIP-2, and transcription of its target genes, including infection response gene-1 (irg-1), at least in the nematode, *C. elegans*. Other key host perturbations may include pathogen

driven rearrangements of the cytoskeleton and it is possible that some of the cytosolic PRRs such as the NLRs and DNA sensors may be involved in recognition of these metabolic changes (Vance et al., 2009). Triggering of these cytosolic PRRs adds a further ratcheting up of responses by inducing inflammasome formation and the activation of caspases. These enzymes in turn activate and release the alarmin, interleukin 1β. Inflammatory cell death (necrotic or pyroptotic) (Duprez et al., 2009) leads to the release of other alarmins and up-regulation of type I interferon pathways (Kersse et al., 2011). Thus, as the second and third checkpoints are breached, the level of the inflammatory response increases significantly. Since many of these host processes are part of cellular or tissue homeostasis, and also potentially highly immunopathogenic, one might argue that gene variants encoding the major regulators during these two phases would be prime candidates for "tolerance" genes. However, their identities are only just becoming clearer and many remain controversial.

A further checkpoint is the detection of invasion from compartments that allow colonization, for example the lumen of the gut, into sterile tissues. Such invasion could occur through tissue injury or expression of virulence factors. This transfer into a new host niche exposes invading micro-organisms to the attention of innate immune cells especially, phagocytes including neutrophils, and the myeloid APC, Mφ, and dendritic cells (DC). These are primed for scaling up effector mechanisms including phagocytosis, cell death and release of defense-related molecules. Mφ are perhaps, pivotal cells during this phase as they have an extensive range of effector functions which depending on their microenvironment, include phagocytosis, scavenging, cytotoxicity, and production of pro- and anti-inflammatory cytokines (Gordon and Taylor, 2005; Plueddemann et al., 2011).

In mice and humans various APC subsets have been identified including M1 and M2 Mφ, which are involved in inflammatory signals and tissue repair respectively, but these classifications are less clear in livestock species (Gordon and Taylor, 2005; Fairbairn et al., 2011; Murray and Wynn, 2011). In particular, Mφ and other phagocytes discriminate between host cells that have undergone different types of cell death and produce a range of immunomodulatory molecules that determine, whether inflammation develops or tissue repair occurs (Poon et al., 2010). Thus, Mφ phagocytosis of cells in the early stages of apoptosis tends to result in the production of factors such as vascular endothelial growth factor (VEGF) and transforming growth factor-β (TGF β), which result in tissue repair and down-regulation of inflammation i.e., Mφ function to return and maintain tissue homeostasis. Phagocytosis of cells undergoing apoptosis by DC results in tolerance (as per its immunological definition) to self. In contrast, phagocytosis of cells in the late stages of apoptosis or those that have undergone inflammatory forms of cell death (necrosis or pyroptosis), result in Mφ producing pro-inflammatory cytokines and further immunopathology. However, this is an over-simplification, and Mφ (and DC) express a wide-range of intermediate phenotypes, apart from those associated with M1 and M2 Mφ in response to internal and external purturbations, whether from micro-organisms or intrinsically derived signals.

Thus, the degree of inflammation and other responses is determined by the different temporal and spatial interactions between host and micro-organism. These processes in turn result in recruitment of further immune cells to the site of infection and ultimately induction of the adaptive response, at least in jawed vertebrates.

INITIATION OF HOST ADAPTIVE RESPONSES BY THE INNATE IMMUNE SYSTEM

Most of the studies that have identified that genetic variation in resistance and tolerance exists in animals have been conducted in species that do not have adaptive immune systems (Graham et al., 2011; Ayres and Schneider, 2012). One might argue that disease resistance or tolerance genes would not be functional during the adaptive immune response, because it takes several days for an adaptive immune response to develop as it is dependent on the innate immune response, which both drives and directs the adaptive response. Without an innate response, no adaptive immune response occurs. In addition, the cells and molecules of the adaptive response also influence the innate immune response. Thus, it seems likely that resistance and tolerance traits may also be expressed as part of the link between the innate and acquired immune system. Indeed, the best known of all the immune-related candidates for disease resistance are the highly polymorphic major histocompatibility complex (MHC) classical genes, MHC I and MHC II (Hill, 2012), which are crucial for presentation of pathogen derived antigen to T cells, without which no induction of pathogen specific adaptive immunity would occur. Infectious disease associations with these loci abound in livestock studies (e.g., see reviews on cattle, pigs, and chickens respectively, Lewin et al., 1999; Lunney et al., 2009; Calenge et al., 2010).

The key players in linking the innate to the adaptive immune system are APC, Mφ, and DC (Hume, 2008; Segura and Villadangos, 2009), which directly initiate the adaptive immune response through interactions with T cells. Depending on the microenvironment and pathogen, APC, in conjunction with MHC class I and class II presentation of pathogen derived peptides to T cells, also express different cytokines and other cell surface molecules which provide the context or second signals that result in the activation of functionally distinct subsets of T cells. Thus, initiation of an adaptive response also relies on the early interactions with pathogens and other intrinsic "danger" signals to provide the second signal along with antigen presentation. As with APC, these T cell subsets have well-studied phenotypes in mouse and human with specific roles in inflammation and host immunity, regulation, and suppression of uncontrolled inflammation (Nakayamada et al., 2012), but are less well-described for livestock.

In brief, intra-cellular infectious agents together with accompanying inflammation invoke the classical inflammatory M1 Mφ to produce IL12 and IL23, which provide the second signals that prime Th1 and Th17 cells respectively. These cells which are involved in amplifying the functions of and induction of cytotoxic T cells through the actions of their signature cytokines, interferon-γ (IFNγ) and interleukin (IL)-17 respectively. However, Th17 cells which reside in the epithelial layers are not only pro-inflammatory, they also help to restore barrier

function following inflammation through their production of the tissue protectant IL-22 (Ouyang et al., 2011; Akdis et al., 2012). Since M1 cells are also major producers of pro-inflammatory cytokines and ROS, they also have the potential to cause tissue damage.

In contrast, extracellular and metazoan pathogens invoke M2-induced Th2 cells, which produce B cell help factors such as IL4 and IL13. In addition, Th2 cells (together with M2 Mφ) also have a regulatory role in limiting and resolving Th1 type inflammation, a role in tissue repair and potentially a role in tolerating the presence of metazoan micro-organisms (Martinez et al., 2009; Allen and Wynn, 2011). Although mouse strains can differ in their propensity to develop M1 vs. M2 response phenotypes (Mills et al., 2000), there has been very little investigation into the identification of the underlying gene variants. One recent paper has suggested that polymorphisms in the transcription factor, interferon regulatory factor 5, which lead to autoimmunity, may be linked to its role in promoting the induction of M1 cells (Krausgruber et al., 2011). Although it might be tempting to suggest that genetic variants which predispose animals to make an M2/Th2 associated response might enhance their tolerant phenotypes, in fact these cell types are also inflammatory (Jenkins et al., 2011). Furthermore, mouse strains with a Th2 type propensity to respond to pathogens are more susceptible to pathogens that require a Th1 type response for adequate protection (Mills et al., 2000). Nevertheless, further understanding of genetics which may underlie a predisposition to macrophage polarization into distinct phenotypes may be very instructive for influencing the genetics of resistance and tolerance to prevailing micro-organisms in livestock species.

HOST ADAPTIVE IMMUNE RESPONSES

Apart from interactions between the innate and the adaptive immune systems, it is also possible that resistance and tolerance genes may be associated with the main players in the acquired immune response, namely the T and B cells. Although it has been assumed that somatic recombination of antibody and T cell receptor (TCR) genes generates very high and similar levels of diversity of pathogen recognition in all individuals, genetic differences between individuals remains a possibility. Firstly, the inherent, constitutive or basal level of immunity will also encompass the components of the adaptive immune system (Clapperton et al., 2009) and therefore this must have a separate associated cost to the host organisms, further complicating the picture with respect to life history trade-offs. Secondly, it was argued that the high potential diversity of antibody and TCRs should mean that all individuals in a species would not be limited in terms of recognition of foreign antigen because of "holes in the repertoire," i.e., be unable to respond because of deficiencies in the germline components of antibody or TCR genes, or because of elimination of self-reactive T or B cells (Goodnow, 1996). However, viral escape through mutation of viral T cell epitopes has been described, whereby selection in the host appears to favor the appearance of viral mutations that presumably mimic a self-peptide, thus abrogating T cell responses and leading to chronic persistence of the virus (Wolfl et al., 2008). In contrast, a common deletion in the TCR beta-chain locus in humans actually leads to enhanced responses to a virus (Brennan et al., 2012). Thus, it is possible that differences in the TCR repertoire could account for some of the variation in responsiveness in livestock species.

Several other distinct T cell subsets have been described including various regulatory T cell subsets (Tregs) and these provide a further interacting level of immune response that influences the final outcome of pathogen or other insult (Vignali et al., 2008). Their signature cytokines include IL-10 and TGFβ, which are anti-inflammatory and thus they are important in regulating inflammation, and tolerance.

Clearly the components of the acquired immune system cannot be ignored in terms of the genetics of resistance and tolerance in higher vertebrates. However, although adaptive immunity is specific to the pathogen, the initiating responses are much less discriminatory and are more directed by the type and spatial location of the pathogen.

ANTI-INFLAMMATORY PROCESSES, RESOLUTION, AND TISSUE REPAIR

If this cascade of events results in elimination of the invading micro-organism, then resolution of inflammation will occur, partly because of removal of the stimuli, but also because a set of negative feedback loops are also set in motion that control the extent of inflammation. If elimination of pathogens does not occur the result will be chronic inflammation or even mortality because of an over-whelming cytokine storm (Tisoncik et al., 2012). However, the field of anti-inflammation, repair, and resolution is a growing area of research and many new factors and pathways remain to be discovered.

Resolution includes immunoregulatory and tissue repair pathways, but these are interdependent with the pathways leading to inflammation (Serhan and Savill, 2005; Shields et al., 2011) and their activation may result in a return to homeostasis or more permanent tissue damage such as scarring and fibrosis. An important point is that, many of these resolution and regulatory pathways are triggered by the same receptor generated signals as inflammation itself. In fact, intact PRR signaling has been shown to be essential for maintenance of tissue homeostasis and tissue repair (Lawrence et al., 2001; Rakoff-Nahoum et al., 2004; Jiang et al., 2005). In a mouse model of *Citrobacter rodentium* infection in the gut, Bergstrom et al. (2012) suggest that resistance and tolerance are interlinked through TLR and NLR-based mechanisms. In a *Drosophila* model, a homolog of the MAP kinase family, p38, which conventionally is regarded as part of the signaling process for defense, has been linked to tolerance through a role in phagocytosis (Shinzawa et al., 2009).

Shields et al. (2011) have also proposed that resolution may involve the recognition of resolution-associated molecular patterns (RAMPs). They suggest that these are endogenous molecules expressed and released when cells are necrotic or stressed and include heat shock proteins which are involved in the cellular translocation of proteins, as chaperones in the correct folding of newly synthesized protein in the endoplasmic reticulum and also in targeting proteins for the proteasome for degradation.

Specific down-regulation of inflammation involves the induction of anti-inflammatory molecules such as IL-10, TGFβ, IL-1R

antagonist, suppressors of cytokine signalling (SOCS), peroxisome proliferator-activated receptor (PPAR) ligands, tyrosine phosphatases SH2-containing phosphatase 1 (SHP-1) and many others, at least partly through the induction of Tregs (Straus and Glass, 2007; Yoshimura et al., 2007; Ouyang et al., 2011; Shields et al., 2011). Recent research has suggested that induction of an IL-6 cytokine family member, leukemia inhibitory factor (LIF) leads to STAT3 signaling and may control tissue repair in lungs of mice suffering from pneumonia (Quinton et al., 2012). LIF has previously been associated with stem cell maintenance and this function may be operating in this situation by promoting cell proliferation or limiting cell death, suggesting further avenues for identifying new tolerance genes.

However, resolution of inflammation is not simply an anti-inflammatory suppressive process, but involves an active process in which specific metabolites are biosynthesized from essential fatty acids in epithelial cells and macrophages in response to inflammation. They include lipoxins, resolution-phase interaction products (resolvins), protectins, sphingosine-1-phosphate, and Maresin 1 (Rivera et al., 2008; Serhan et al., 2008, 2012). These mediators induce uptake and clearance of dead cells and pathogens and they stimulate tissue regeneration and are thus important for homeostatic mechanisms. They are synthesized through cyclooxygenase, lipoxygenase, and epoxygenase pathways and many of the intermediates and products of these pathways are anti-inflammatory and act through PPAR transcription factors (Wahli and Michalik, 2012). PPARs signaling down-regulates the inflammatory transcription profile by suppressing the activity of inflammation-responsive transcription factors including NF-kB. They also maintain an M2 Mφ phenotype in tissues, promoting tissue maintenance activities through M2 Mφ role in scavenging, angiogenesis, tissue remodeling, and repair (Martinez et al., 2009). Given the role of PPARs, M1 and M2 cells in adipocyte, fatty acid and energy metabolism (Shapiro et al., 2011), their modulation could have very important bearing on resistance and tolerance traits, but again much remains to be discovered about them.

Thus, it should be emphasized that the mechanisms controlling anti-inflammatory processes, resolution, and tissue repair play an essential role in regulating the host response to invading pathogens at all levels from the initial recognition of pathogen presence to the triggering of the innate and acquired immune mechanisms. The genes involved cannot be easily categorized as simply part of tolerance traits but are intimately linked to resistance traits as well.

IDENTIFICATION OF LOCI AND GENETIC VARIANTS FOR RESISTANCE AND TOLERANCE

So what is the evidence that different genetic variants exist for resistance and tolerance in complex metazoans? Some recent evidence is pertinent and will illustrate the difficulties of consigning genes to one or the other trait.

As previously discussed there is evidence that, there is a genetic component that underlies variation in resistance and tolerance. Although it is difficult to demonstrate directly that hosts and pathogens co-evolve, the general consensus is that evidence for this can be seen in changes in frequencies of genes in populations exposed to specific pathogens (Allison, 1954; De Campos-Lima et al., 1994; Novembre and Han, 2012) and in terms of signals of positive selection in genomes (Fumagalli et al., 2011) with innate immune genes having the strongest signals (Barreiro and Quintana-Murci, 2010). It has to be said that these types of analyses are more difficult when attempting to include non-model species with relatively poor annotation such as livestock (Brieuc and Naish, 2011) with limited data on frequencies of candidate genes in different populations. The author and others have shown that positive selection in immune related genes can be detected, but obtaining evidence that positively selected sites are important functionally in livestock is more difficult (e.g., Jann et al., 2008; Smith et al., 2012). In addition, most if not all studies on candidates concentrate on defense, and not on tolerance. Thus, it remains to be seen if similar differences in frequencies or evidence of positive selection can be found for livestock or indeed any other population.

The most convincing example of pathogen driven selection of host genes, is the interaction of human populations with *Plasmodium falciparum* where frequencies of gene variants associated with malaria resistance and tolerance are at significantly higher levels in areas where malaria is prevalent than in non-endemic parts of the world (Durand and Coetzer, 2008). Thus, in the case of malaria, homozygosity for the hemoglobin mutation that causes sickle cell anemia (HbS), is associated with loss of fitness or reproductive success, which acts as a counter balance, preventing the sickle cell gene from going to fixation in a population where malaria is endemic (Allison, 1954). Recently, it has become apparent that the HbS gene confers both resistance and tolerance to the malaria parasite (Ferreira et al., 2011). In addition, Seixas et al. (2009) have identified a mechanism that confers tolerance to malaria, which involves the induction of heme oxygenase-1 (HO-1) in the liver of a tolerant, but not in a susceptible mouse strain. HO-1 is a tissue protectant which degrades free heme released from hemoglobin in *Plasmodium* infected red cells. Free heme leads to the production of ROS, resulting in apoptosis of liver cells. The underlying genetics that leads to differential HO-1 expression in more or less tolerant mouse strains remains unclear. However, in humans, polymorphism in the promoter region of HO-1 controls its expression and is associated with severe malaria (Walther et al., 2012). Again, whilst it might seem attractive to target HO-1 as a potential candidate gene for tolerance, this may be counterproductive as induction of HO-1 during malaria infection resulted in a fatal reduction in resistance to *Salmonella* in mice (Cunnington et al., 2012).

In a unique study, Miyairi et al. (2012) have specifically investigated the genetics of resistance and tolerance to *Chlamydia psittaci* in a range of genotyped mice. The authors measured weight loss and pathogen burden following infection and identified a genetic locus on mouse chromosome 11 (*Ctrq3*), which influenced pathogen load i.e., resistance as well as weight loss and thus suggest that the same locus controls resistance and tolerance. They have circumstantial evidence that likely candidate genes belong to the family of immunity-related GTPases (IRG). These genes encode proteins which are highly up-regulated in pathogen containing cells such as macrophages (Taylor et al., 2007).

They localize to pathogen containing vacuoles and are involved in processing of pathogens through destructive pathways such as autophagy resulting in the elimination of the pathogen. Bearing in mind that the authors measured a limited number of traits, they presented evidence that the *Ctrq3* locus also controls macrophage activation and neutrophil accumulation at the site of infection, but these traits also independently influenced weight loss. Macrophage activation also had an independent effect on pathogen burden. These intriguing results again point to the importance of macrophages in determining tolerance and resistance traits.

As discussed previously in livestock species even where appropriate parameters have been measured, their relationships have not been explored. An exception is a paper by Zanella et al. (2011) who have defined loci for tolerance to infection with *Mycobacterium avium* subspecies *paratuberculosis* (MAP), by measuring MAP fecal shedding as a measure of fitness and MAP levels in tissues as a measure of infection intensity. It may be instructive to revisit previously published data to explore the components of resistance and tolerance in livestock responses to pathogens. For example, in the author's own research a *Bos indicus* cattle breed, Sahiwal, has been shown to be more resistant to a tropical tick-borne protozoan parasite, *Theileria annulata* than a non-tropical breed, the Holstein (Glass et al., 2005). Both breeds became infected following experimental challenge, but only the Sahiwals survived to the end of the experiment, the Holsteins being overcome with an overwhelming inflammatory response. The parasite infects Mφ which also plays an important role in the inflammatory response as well as protection against the parasite. Among other transcriptional differences between the two breeds (Jensen et al., 2008), two molecules stand out: signal regulatory protein beta (SIRPβ), and TGFβ2 (Chaussepied et al., 2010; Glass et al., 2012b). Both are involved in regulation of inflammation and both were more highly expressed in Holstein Mφ than Sahiwal Mφ. TGFβ2 appears to be associated with greater virulence and also higher propensity for invasion (Chaussepied et al., 2010). Given these intriguing differences, reconsideration of the original data in terms of regressing health against pathogen levels in the two breeds, is warranted as the pathogen burden was less in the Sahiwals, and some parameters of fitness (temperature and packed cell volume) and clinical, hematological, and inflammatory related responses were measured before and during the experimental trial.

CONCLUSIONS

In summary, although genetic resistance and tolerance are likely underpinned by distinct mechanisms, their initiation is likely to be intertwined and the outcome of host-pathogen interactions is dependent on both the host and pathogen characteristics. Pathogens have evolved very distinct strategies to ensure their reproductive success, which is dependent on their ability to thrive in their host species and to transmit to other individuals. Some pathogens produce factors which cause toxicity in their host, or induce a dysregulated inflammatory response which can prove fatal. Host metazoans that can overcome such pathogens, and survive may adopt two distinct strategies, first: quickly eliminate such pathogens; second, tolerate the effects by producing antitoxins for example, or employ stronger or faster acting negative feedback loops to prevent inflammation damaging the tissues. Although these strategies appear to be distinct, in fact the complex host processes encompassing these tactics are intimately entwined. Thus, distinct host resistance and tolerance traits may be less common than traits that involve elements of both strategies which are likely to have evolved together to overcome infectious threats. Pathogens can also manipulate the host response to their own gain, for example driving recruitment of immune cells that provide cellular niches for the dissemination of the pathogens. Pathogens have also evolved strategies to overcome, evade and subvert the host defense mechanisms, and instead of their removal, infection may become chronic; such a scenario may result in reduction in fitness of the host, but is not inevitable. Much remains to be discovered especially the genes and pathways controlling anti-inflammatory responses, resolution and tissue repair. A better understanding of these mechanisms and their relationship to inflammation and the pathogen driven host defense responses, especially in livestock species, is clearly needed before we can begin to breed livestock for increased resistance and/or tolerance.

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REFERENCES

Ahrens, S., Zelenay, S., Sancho, D., Hanc, P., Kjaer, S., Feest, C., et al. (2012). F-actin is an evolutionarily conserved damage-associated molecular pattern recognized by DNGR-1, a receptor for dead cells. *Immunity* 36, 635–645.

Akdis, M., Palomares, O., van de Veen, W., van Splunter, M., and Akdis, C. A. (2012). T(H)17 and T(H)22 cells: a confusion of antimicrobial response with tissue inflammation versus protection. *J. Allergy Clin. Immunol.* 129, 1438–1449.

Alizon, S., Hurford, A., Mideo, N., and Van Baalen, M. (2009). Virulence evolution and the trade-off hypothesis: history, current state of affairs and the future. *J. Evol. Biol.* 22, 245–259.

Allen, J. E., and Wynn, T. A. (2011). Evolution of Th2 immunity: a rapid repair response to tissue destructive pathogens. *PLoS Pathog.* 7:e1002003. doi: 10.1371/journal.ppat.1002003

Allison, A. C. (1954). Protection afforded by sickle-cell trait against subtertian malarial infection. *Br. Med. J.* 1, 290–294.

Ayres, J. S., and Schneider, D. S. (2008). A signaling protease required for melanization in Drosophila affects resistance and tolerance of infections. *PLoS Biol.* 6:e305. doi: 10.1371/journal.pbio.0060305

Ayres, J. S., and Schneider, D. S. (2009). The role of anorexia in resistance and tolerance to infections in Drosophila. *PLoS Biol.* 7:e1000150. doi: 10.1371/journal.pbio.1000150

Ayres, J. S., and Schneider, D. S. (2012). Tolerance of infections. *Annu. Rev. Immunol.* 30, 271–294.

Barreiro, L. B., and Quintana-Murci, L. (2010). From evolutionary genetics to human immunology: how selection shapes host defence genes. *Nat. Rev. Genet.* 11, 17–30.

Bergstrom, K. S., Sham, H. P., Zarepour, M., and Vallance, B. A. (2012). Innate host responses to enteric bacterial pathogens: a balancing act between resistance and tolerance. *Cell. Microbiol.* 14, 475–484.

Best, A., Long, G., White, A., and Boots, M. (2012). The implications of immunopathology for parasite evolution. *Proc. R. Soc. B Biol. Sci.* 279, 3234–3240.

Bianchi, M. E. (2007). DAMPs, PAMPs and alarmins: all we need to know about danger. *J. Leukoc. Biol.* 81, 1–5.

Blanchet, S., Rey, O., and Loot, G. (2010). Evidence for host variation in parasite tolerance in a wild fish population. *Evol. Ecol.* 24, 1129–1139.

Blander, J. M., and Sander, L. E. (2012). Beyond pattern recognition: five immune checkpoints for scaling the microbial threat. *Nat. Rev. Immunol.* 12, 215–225.

Brennan, R. M., Petersen, J., Neller, M. A., Miles, J. J., Burrows, J. M., Smith, C., et al. (2012). The impact of a large and frequent deletion in the human tcr beta locus on antiviral immunity. *J. Immunol.* 188, 2742–2748.

Brieuc, M. S. O., and Naish, K. A. (2011). Detecting signatures of positive selection in partial sequences generated on a large scale: pitfalls, procedures and resources. *Mol. Ecol. Resour.* 11, 172–183.

Calenge, F., Kaiser, P., Vignal, A., and Beaumont, C. (2010). Genetic control of resistance to salmonellosis and to Salmonella carrier-state in fowl: a review. *Genet. Sel. Evol.* 42, 11.

Carval, D., and Ferriere, R. (2010). A unified model for the coevolution of resistance, tolerance, and virulence. *Evolution* 64, 2988–3009.

Casadevall, A., and Pirofski, L. A. (2001). Host-pathogen interactions: the attributes of virulence. *J. Infect. Dis.* 184, 337–344.

Chaussepied, M., Janski, N., Baumgartner, M., Lizundia, R., Jensen, K., Weir, W., et al. (2010). TGF-β2 induction regulates invasiveness of *Theileria* transformed leukocytes and disease susceptibility. *PLoS Pathog.* 6:e1001197. doi: 10.1371/journal.ppat.1001197

Clapperton, M., Diack, A. B., Matika, O., Glass, E. J., Gladney, C. D., Mellencamp, M. A., et al. (2009). Traits associated with innate and adaptive immunity in pigs: heritability and associations with performance under different health status conditions. *Genet. Sel. Evol.* 41, 54.

Clermont, O., Olier, M., Hoede, C., Diancourt, L., Brisse, S., Keroudean, M., et al. (2011). Animal and human pathogenic *Escherichia coli* strains share common genetic backgrounds. *Infect. Genet. Evol.* 11, 654–662.

Corby-Harris, V., Habel, K. E., Ali, F. G., and Promislow, D. E. L. (2007). Alternative measures of response to *Pseudomonas aeruginosa* infection in *Drosophila melanogaster*. *J. Evol. Biol.* 20, 526–533.

Cunnington, A. J., de Souza, J. B., Walther, M., Riley, E. M. (2012). Malaria impairs resistance to Salmonella through heme- and heme oxygenase-dependent dysfunctional granulocyte mobilization. *Nat. Med.* 18, 120–127.

Davies, G., Genini, S., Bishop, S. C., and Giuffra, E. (2009). An assessment of opportunities to dissect host genetic variation in resistance to infectious diseases in livestock. *Animal* 3, 415–436.

Day, T., Graham, A. L., and Read, A. F. (2007). Evolution of parasite virulence when host responses cause disease. *Proc. R. Soc. B Biol. Sci.* 274, 2685–2692.

Dean, M., Carrington, M., and O'Brien, S. J. (2002). Balanced polymorphism selected by genetic versus infectious human disease. *Annu. Rev. Genomics Hum. Genet.* 3, 263–292.

De Campos-Lima, P. O., Levitsky, V., Brooks, J., Lee, S. P., Hu, L. F., Dickinson, A. B., et al. (1994). T-Cell responses and virus evolution -loss of HLA A11-restricted CTL epitopes in Epstein-Barr-Virus isolates from highly A11-positive populations by selective mutation of anchor residues. *J. Exp. Med.* 179, 1297–1305.

Doeschl-Wilson, A. B., Bishop, S. C., Kyriazakis, I., and Villanueva, B. (2012a). Two methods for quantifying individual host response to infectious pathogens in terms of infection severity and impact. *Front. Gene.* 3:266. doi: 10.3389/fgene.2012.00266

Doeschl-Wilson, A. B., Villanueva, B., and Kyriazakis, I. (2012b). The first step towards genetic selection for host tolerance to infectious pathogens: obtaining the tolerance phenotype through group estimates. *Front. Gene.* 3:265. doi: 10.3389/fgene.2012.00265

Duprez, L., Wirawan, E., Vanden Berghe, T., and Vandenabeele, P. (2009). Major cell death pathways at a glance. *Microbes Infect.* 11, 1050–1062.

Durand, P. M., and Coetzer, T. L. (2008). Hereditary red cell disorders and malaria resistance. *Haematologica* 93, 961–963.

Ebert, D., and Bull, J. J. (2003). Challenging the trade-off model for the evolution of virulence: is virulence management feasible? *Trends Microbiol.* 11, 15–20.

Evans, S. E., Scott, B. L., Clement, C. G., Larson, D. T., Kontoyiannis, D., Lewis, R. E., et al. (2010). Stimulated innate resistance of lung epithelium protects mice broadly against bacteria and fungi. *Am. J. Respir. Cell Mol. Biol.* 42, 40–50.

Fairbairn, L., Kapetanovic, R., Sester, D. P., and Hume, D. A. (2011). The mononuclear phagocyte system of the pig as a model for understanding human innate immunity and disease. *J. Leukoc. Biol.* 89, 855–871.

Ferreira, A., Marguti, I., Bechmann, I., Jeney, V., Chora, A., Palha, N. R., et al. (2011). Sickle hemoglobin confers tolerance to plasmodium infection. *Cell* 145, 398–409.

Fontana, M. F., and Vance, R. E. (2011). Two signal models in innate immunity. *Immunol. Rev.* 243, 26–39.

Fumagalli, M., Sironi, M., Pozzoli, U., Ferrer-Admettla, A., Pattini, L., and Nielsen, R. (2011). Signatures of environmental genetic adaptation pinpoint pathogens as the main selective pressure through human evolution. *PLoS Genet.* 7:e1002355. doi: 10.1371/journal.pgen.1002355

Glass, E. J., Baxter, R., Leach, R. L., and Jann, O. C. (2012a). Genes controlling vaccine responses and disease resistance to respiratory viral pathogens in cattle. *Vet. Immunol. Immunopathol.* 148, 90–99.

Glass, E. J., Crutchley, S., and Jensen, K. (2012b). Living with the enemy or uninvited guests: functional genomics approaches to investigating host resistance or tolerance traits to a protozoan parasite in cattle. *Vet. Immunol. Immunopathol.* 148, 178–189.

Glass, E. J., Baxter, R., Leach, R., and Taylor, G. (2010). "Bovine viral diseases – the role of host genetics," in *Breeding for Disease Resistance in Farm Animals, 3rd Edn.* eds S. C. Bishop, R. F. E. Axford, F. W. Nicholas, and J. B. Owen (Wallingford, UK: CAB International Nosworthy Way), 88–140.

Glass, E. J., Preston, P. M., Springbett, A., Craigmile, S., Kirvar, E., Wilkie, G., et al. (2005). Bos taurus and Bos indicus (Sahiwal) calves respond differently to infection with *Theileria annulata* and produce markedly different levels of acute phase proteins. *Int. J. Parasitol.* 35, 337–347.

Goodnow, C. C. (1996). Balancing immunity and tolerance: deleting and tuning lymphocyte repertoires. *Proc. Natl. Acad. Sci. U.S.A.* 93, 2264–2271.

Gordon, S., and Taylor, P. R. (2005). Monocyte and macrophage heterogeneity. *Nat. Rev. Immunol.* 5, 953–964.

Graham, A. L., Hayward, A. D., Watt, K. A., Pilkington, J. G., Pemberton, J. M., and Nussey, D. H. (2010). Fitness correlates of heritable variation in antibody responsiveness in a wild mammal. *Science* 330, 662–665.

Graham, A. L., Shuker, D. M., Pollitt, L. C., Auld, S. K. J. R., Wilson, A. J., and Little, T. J. (2011). Fitness consequences of immune responses: strengthening the empirical framework for ecoimmunology. *Funct. Ecol.* 25, 5–17.

Hansen, J. D., Vojtech, L. N., and Laing, K. J. (2011). Sensing disease and danger: a survey of vertebrate PRRs and their origins. *Dev. Comp. Immunol.* 35, 886–897.

Hill, A. V. S. (2012). Evolution, revolution and heresy in the genetics of infectious disease susceptibility. *Philos. Trans. R. Soc. B Biol. Sci.* 367, 840–849.

Hume, D. A. (2008). Macrophages as APC and the dendritic cell myth. *J. Immunol.* 181, 5829–5835.

Hume, D. A., Whitelaw, C. B. A., and Archibald, A. L. (2011). The future of animal production: improving productivity and sustainability. *J. Agric. Sci.* 149, 9–16.

Janeway, C. A. (1989). Approaching the asymptote – evolution and revolution in immunology. *Cold Spring Harb. Symp. Quant. Biol.* 54, 1–13.

Jann, O. C., King, A., Corrales, N. L., Anderson, S. I., Jensen, K., Ait-ali, T., et al. (2009). Comparative genomics of Toll-like receptor signalling in five species. *BMC Genomics* 10:216. doi: 10.1186/1471-2164-10-216

Jann, O. C., Werling, D., Chang, J. S., Haig, D., and Glass, E. J. (2008). Molecular evolution of bovine Toll-like receptor 2 suggests substitutions of functional relevance. *BMC Evol. Biol.* 8:288. doi: 10.1186/1471-2148-8-288

Jenkins, S. J., Ruckerl, D., Cook, P. C., Jones, L. H., Finkelman, F. D., van Rooijen, N., et al. (2011). Local macrophage proliferation, rather than recruitment from the blood, is a signature of T(h)2 inflammation. *Science* 332, 1284–1288.

Jensen, K., Paxton, E., Waddington, D., Talbot, R., Darghouth, M. A., and Glass, E. J. (2008). Differences in the transcriptional responses induced by *Theileria annulata* infection in bovine monocytes derived from resistant and susceptible cattle breeds. *Int. J. Parasitol.* 38, 313–325.

Jiang, D. H., Liang, J. R., Fan, J., Yu, S., Chen, S. P., Luo, Y., et al. (2005). Regulation of lung injury and repair by Toll-like receptors and hyaluronan. *Nat. Med.* 11, 1173–1179.

Kawai, T., and Akira, S. (2011). Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. *Immunity* 34, 637–650.

Kersse, K., Bertrand, M. J. M., Lamkanfi, M., and Vandenabeele, P. (2011). NOD-like receptors and the innate immune system: coping with danger, damage and death. *Cytokine Growth Factor Rev.* 22, 257–276.

Kirchner, J. W., and Roy, B. A. (2002). Evolutionary implications of host-pathogen specificity: fitness consequences of pathogen virulence traits. *Evol. Ecol. Res.* 4, 27–48.

Kleino, A., and Silverman, N. (2012). UnZIPping mechanisms of effector-triggered immunity in animals. *Cell Host Microbe* 11, 320–322.

Krausgruber, T., Blazek, K., Smallie, T., Alzabin, S., Lockstone, H., Sahgal, N., et al. (2011). IRF5 promotes inflammatory macrophage polarization and T(H)1-T(H)17 responses. *Nat. Immunol.* 12, 231–238.

Kumar, H., Kawai, T., and Akira, S. (2011). Pathogen recognition by the innate immune system. *Int. Rev. Immunol.* 30, 16–34.

Lawrence, T., Gilroy, D. W., Colville-Nash, P. R., and Willoughby, D. A. (2001). Possible new role for NF-kappa B in the resolution of inflammation. *Nat. Med.* 7, 1291–1297.

Lazzaro, B. P., and Little, T. J. (2009). Immunity in a variable world. *Philos. Trans. R. Soc. B Biol. Sci.* 364, 15–26.

Leach, R. J., O'Neill, R. G., Fitzpatrick, J. L., Williams, J. L., and Glass, E. J. (2012). Quantitative trait loci associated with the immune response to a bovine respiratory syncytial virus vaccine. *PLoS ONE* 7:e33526. doi: 10.1371/journal.pone.0033526

Lefevre, T., Williams, A. J., and de Roode, J. C. (2011). Genetic variation in resistance, but not tolerance, to a protozoan parasite in the monarch butterfly. *Proc. R. Soc. B Biol. Sci.* 278, 751–759.

Lewin, H. A., Russell, G. C., and Glass, E. J. (1999). Comparative organization and function of the major histocompatibility complex of domesticated cattle. *Immunol. Rev.* 167, 145–158.

Lotze, M. T., Zeh, H. J., Rubartelli, A., Sparvero, L. J., Amoscato, A. A., Washburn, N. R., et al. (2007). The grateful dead: damage-associated molecular pattern molecules and reduction/oxidation regulate immunity. *Immunol. Rev.* 220, 60–81.

Lunney, J. K., Ho, C. S., Wysocki, M., and Smith, D. M. (2009). Molecular genetics of the swine major histocompatibility complex, the SLA complex. *Dev. Comp. Immunol.* 33, 362–374.

Manson, J., Thiemermann, C., and Brohi, K. (2012). Trauma alarmins as activators of damage-induced inflammation. *Br. J. Surg.* 99, 12–20.

Margolis, E., and Levin, B. R. (2008). "Evolution of bacterial-host interactions: virulence and the immune overresponse," in *Evolutionary Biology of Bacterial and Fungal Pathogens,* eds F. Baquero, C. Nombela, G. Cassell, and J. Gutierrez Fuentes (Washington, DC: American Society for Microbiology), 3–12.

Marsh, E. K., van den Berg, M. C. W., and May, R. C. (2011). A two-gene balance regulates *Salmonella typhimurium* tolerance in the nematode *Caenorhabditis elegans*. *PLoS ONE* 6:e16839. doi: 10.1371/journal.pone.0016839

Martinez, F. O., Helming, L., and Gordon, S. (2009). Alternative activation of macrophages: an immunologic functional perspective. *Annu. Rev. Immunol.* 27, 451–483.

Matzinger, P. (1994). Tolerance, danger, and the extended family. *Annu. Rev. Immunol.* 12, 991–1045.

Matzinger, P. (2002). The danger model: a renewed sense of self. *Science* 296, 301–305.

Matzinger, P. (2007). Friendly and dangerous signals: is the tissue in control? *Nat. Immunol.* 8, 11–13.

Maurer, K., Krey, T., Moennig, V., Thiel, H. R., and Rumenapf, T. (2004). CD46 is a cellular receptor for bovine viral diarrhea virus. *J. Virol.* 78, 1792–1799.

Medzhitov, R. (2009). Damage control in host-pathogen interactions. *Proc. Natl. Acad. Sci. U.S.A.* 106, 15525–15526.

Meijerink, E., Fries, R., Vogeli, P., Masabanda, J., Wigger, G., Stricker, C., et al. (1997). Two alpha(1, 2) fucosyltransferase genes on porcine Chromosome 6q11 are closely linked to the blood group inhibitor (S) and Escherichia coli F18 receptor (ECF18R) loci. *Mamm. Genome* 8, 736–741.

Mills, C. D., Kincaid, K., Alt, J. M., Heilman, M. J., and Hill, A. M. (2000). M-1/M-2 macrophages and the Th1/Th2 paradigm. *J. Immunol.* 164, 6166–6173.

Mirkena, T., Duguma, G., Haile, A., Tibbo, M., Okeyo, A. M., Wurzinger, M., et al. (2010). Genetics of adaptation in domestic farm animals: a review. *Livest. Sci.* 132, 1–12.

Miyairi, I., Ziebarth, J., Laxton, J. D., Wang, X. F., van Rooijen, N., Williams, R. W., et al. (2012). Host genetics and Chlamydia disease: prediction and validation of disease severity mechanisms. *PLoS ONE* 7:e33781. doi: 10.1371/journal.pone.0033781

Monaghan, P., Gold, S., Simpson, J., Zhang, Z. D., Weinreb, P. H., Violette, S. M., et al. (2005). The alpha v beta 6 integrin receptor for Foot-and-mouth disease virus is expressed constitutively on the epithelial cells targeted in cattle. *J. Gen. Virol.* 86, 2769–2780.

Murray, P. J., and Wynn, T. A. (2011). Protective and pathogenic functions of macrophage subsets. *Nat. Rev. Immunol.* 11, 723–737.

Nakayamada, S., Takahashi, H., Kanno, Y., and O'Shea, J. J. (2012). Helper T cell diversity and plasticity. *Curr. Opin. Immunol.* 24, 297–302.

Namangala, B. (2012). Contribution of innate immune responses towards resistance to African trypanosome infections. *Scand. J. Immunol.* 75, 5–15.

Novembre, J., and Han, E. J. (2012). Human population structure and the adaptive response to pathogen-induced selection pressures. *Philos. Trans. R. Soc. B Biol. Sci.* 367, 878–886.

Noyes, H., Brass, A., Obara, I., Anderson, S., Archibald, A. L., Bradley, D. G., et al. (2011). Genetic and expression analysis of cattle identifies candidate genes in pathways responding to *Trypanosoma congolense* infection. *Proc. Natl. Acad. Sci. U.S.A.* 108, 9304–9309.

Nussbaum, J. C., and Locksley, R. M. (2012). Infectious (non)tolerance-frustrated commensalism gone awry? *Cold Spring Harb. Perspect. Biol.* 4:a007328. doi: 10.1101/cshperspect.a007328

Ouyang, W. J., Rutz, S., Crellin, N. K., Valdez, P. A., and Hymowitz, S. G. (2011). Regulation and functions of the IL-10 family of cytokines in inflammation and disease. *Annu. Rev. Immunol.* 29, 71–109.

Pamer, E. G. (2007). Immune responses to commensal and environmental microbes. *Nat. Immunol.* 8, 1173–1178.

Plueddemann, A., Mukhopadhyay, S., and Gordon, S. (2011). Innate immunity to intracellular pathogens: macrophage receptors and responses to microbial entry. *Immunol. Rev.* 240, 11–24.

Poon, I. K. H., Hulett, M. D., and Parish, C. R. (2010). Molecular mechanisms of late apoptotic/necrotic cell clearance. *Cell Death Differ.* 17, 381–397.

Poulin, R., and Combes, C. (1999). The concept of virulence: interpretations and implications – comment. *Parasitol. Today* 15, 474–475.

Quinton, L. J., Mizgerd, J. P., Hilliard, K. L., Jones, M. R., Kwon, C. Y., and Allen, E. (2012). Leukemia inhibitory factor signaling is required for lung protection during pneumonia. *J. Immunol.* 188, 6300–6308.

Raberg, L., Graham, A. L., and Read, A. F. (2009). Decomposing health: tolerance and resistance to parasites in animals. *Philos. Trans. R. Soc. B Biol. Sci.* 364, 37–49.

Raberg, L., Sim, D., and Read, A. F. (2007). Disentangling genetic variation for resistance and tolerance to infectious diseases in animals. *Science* 318, 812–814.

Rakoff-Nahoum, S., Paglino, J., Eslami-Varzaneh, F., Edberg, S., and Medzhitov, R. (2004). Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. *Cell* 118, 229–241.

Restif, O., and Koella, J. C. (2004). Concurrent evolution of resistance and tolerance to pathogens. *Am. Nat.* 164, E90–E102.

Reynes, J., Portales, P., Segondy, M., Baillat, V., Andre, P., Avinens, O., et al. (2001). CD4 T cell surface CCR5 density as a host factor in HIV-1 disease progression. *AIDS* 15, 1627–1634.

Rivera, J., Proia, R. L., and Olivera, A. (2008). The alliance of sphingosine-1-phosphate and its receptors in immunity. *Nat. Rev. Immunol.* 8, 753–763.

Ronald, P. C., and Beutler, B. (2010). Plant and animal sensors of conserved microbial signatures. *Science* 330, 1061–1064.

Roy, B. A., and Kirchner, J. W. (2000). Evolutionary dynamics of pathogen resistance and tolerance. *Evolution* 54, 51–63.

Schmid-Hempel, P. (2009). Immune defence, parasite evasion strategies and their relevance for 'macroscopic phenomena' such as virulence. *Philos. Trans. R. Soc. B Biol. Sci.* 364, 85–98.

Schneider, D. S. (2011). Tracing personalized health curves during

infections. *PLoS Biol.* 9:e1001158. doi: 10.1371/journal.pbio.1001158
Segura, E., and Villadangos, J. A. (2009). Antigen presentation by dendritic cells *in vivo*. *Curr. Opin. Immunol.* 21, 105–110.
Seixas, E., Gozzelino, R., Chora, A., Ferreira, A., Silva, G., Larsen, R., et al. (2009). Heme oxygenase-1 affords protection against noncerebral forms of severe malaria. *Proc. Natl. Acad. Sci. U.S.A.* 106, 15837–15842.
Seong, S. Y., and Matzinger, P. (2004). Hydrophobicity: an ancient damage-associated molecular pattern that initiates innate immune responses. *Nat. Rev. Immunol.* 4, 469–478.
Serhan, C. N., Chiang, N., and Van Dyke, T. E. (2008). Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. *Nat. Rev. Immunol.* 8, 349–361.
Serhan, C. N., Dalli, J., Karamnov, S., Choi, A., Park, C. K., Xu, Z. Z., et al. (2012). Macrophage proresolving mediator maresin 1 stimulates tissue regeneration and controls pain. *FASEB J.* 26, 1755–1765.
Serhan, C. N., and Savill, J. (2005). Resolution of inflammation: the beginning programs the end. *Nat. Immunol.* 6, 1191–1197.
Shapiro, H., Lutaty, A., and Ariel, A. (2011). Macrophages, meta-inflammation, and immunometabolism. *Sci. World J.* 11, 2509–2529.
Shields, A. M., Panayi, G. S., and Corrigall, V. M. (2011). Resolution-associated molecular patterns (RAMP): RAMParts defending immunological homeostasis? *Clin. Exp. Immunol.* 165, 292–300.
Shinzawa, N., Nelson, B., Aonuma, H., Okado, K., Fukumoto, S., Miura, M., et al. (2009). p38 MAPK-dependent phagocytic encapsulation confers infection tolerance in Drosophila. *Cell Host Microbe* 6, 244–252.
Simms, E. L., and Triplett, J. (1995). Costs and benefits of plant responses to disease: resistance and tolerance. *Evolution* 48, 1973–1985.
Smith, S. A., Jann, O. C., Haig, D., Russell, G. C., Werling, D., Glass, E. J., et al. (2012). Adaptive evolution of Toll-like receptor 5 in domesticated mammals. *BMC Evol. Biol.* 12:122. doi: 10.1186/1471-2148-12-122
Stevens, M. P., Humphrey, T. J., and Maskell, D. J. (2009). Molecular insights into farm animal and zoonotic Salmonella infections. *Philos. Trans. R. Soc. B Biol. Sci.* 364, 2709–2723.
Stjernman, M., Raberg, L., and Nilsson, J. A. (2008). Maximum host survival at intermediate parasite infection intensities. *PLoS ONE* 3:e2463. doi: 10.1371/journal.pone.0002463
Straus, D. S., and Glass, C. K. (2007). Anti-inflammatory actions of PPAR ligands: new insights on cellular and molecular mechanisms. *Trends Immunol.* 28, 551–558.
Swiatczak, B., Rescigno, M., and Cohen, I. R. (2011). Systemic features of immune recognition in the gut. *Microbes Infect.* 13, 983–991.
Taylor, G. A., Feng, C. G., and Sher, A. (2007). Control of IFN-gamma-mediated host resistance to intracellular pathogens by immunity-related GTPases (p47 GTPases). *Microbes Infect.* 9, 1644–1651.
Tisoncik, J. R., Korth, M. J., Simmons, C. P., Farrar, J., Martin, T. R., and Katze, M. G. (2012). Into the eye of the cytokine storm. *Microbiol. Mol. Biol. Rev.* 76, 16–32.
Trivedi, K., Tang, C. M., and Exley, R. M. (2011). Mechanisms of meningococcal colonisation. *Trends Microbiol.* 19, 456–463.
Valarcher, J. F., and Taylor, G. (2007). Bovine respiratory syncytial virus infection. *Vet. Res.* 38, 153–180.
Vance, R. E., Isberg, R. R., and Portnoy, D. A. (2009). Patterns of pathogenesis: discrimination of pathogenic and nonpathogenic microbes by the innate immune system. *Cell Host Microbe* 6, 10–21.
Vanhollebeke, B., and Pays, E. (2010). The trypanolytic factor of human serum: many ways to enter the parasite, a single way to kill. *Mol. Microbiol.* 76, 806–814.
Vignali, D. A., Collison, L. W., and Workman, C. J. (2008). How regulatory T cells work. *Nat. Rev. Immunol.* 8, 523–532.
Viney, M. E., Riley, E. M., and Buchanan, K. L. (2005). Optimal immune responses: immunocompetence revisited. *Trends Ecol. Evol.* 20, 665–669.
Wadhwa, A., Hickling, G. J., and Eda, S. (2012). Opportunities for improved serodiagnosis of human tuberculosis, bovine tuberculosis, and paratuberculosis. *Vet. Med. Int.* 2012:674238. doi: 10.1155/2012/674238
Wahli, W., and Michalik, L. (2012). PPARs at the crossroads of lipid signaling and inflammation. *Trends Endocrinol. Metab.* 23, 351–363.
Walther, M., De Caul, A., Aka, P., Njie, M., Amambua-Ngwa, A., Walther, B., et al. (2012). HMOX1 gene promoter alleles and high HO-1 levels are associated with severe malaria in Gambian children. *PLoS Pathog.* 8:e1002579. doi: 10.1371/journal.ppat.1002579
Werling, D., Jann, O. C., Offord, V., Glass, E. J., and Coffey, T. J. (2009). Variation matters: TLR structure and species-specific pathogen recognition. *Trends Immunol.* 30, 124–130.
Willer, Y., Muller, B., and Bumann, D. (2012). Intestinal inflammation responds to microbial tissue load independent of pathogen/non-pathogen discrimination. *PLoS ONE* 7:e35992. doi: 10.1371/journal.pone.0035992
Wolfl, M., Rutebemberwa, A., Mosbruger, T., Mao, Q., Li, H. M., Netski, D., et al. (2008). Hepatitis C Virus immune escape via exploitation of a hole in the T cell repertoire. *J. Immunol.* 181, 6435–6446.
Woolhouse, M. E. J., Webster, J. P., Domingo, E., Charlesworth, B., and Levin, B. R. (2002). Biological and biomedical implications of the co-evolution of pathogens and their hosts. *Nat. Genet.* 32, 569–577.
Yoshimura, A. Y. K., Naka, T., and Kubo, M. (2007). SOCS proteins, cytokine signalling and immune regulation. *Nat. Rev. Immunol.* 7, 454–465.
Zanella, R., Settles, M. L., McKay, S. D., Schnabel, R., Taylor, J., Whitlock, R. H., et al. (2011). Identification of loci associated with tolerance to Johne's disease in Holstein cattle. *Anim. Genet.* 42, 28–38.
Zarember, K. A., and Godowski, P. J. (2002). Tissue expression of human toll-like receptors and differential regulation of toll-like receptor mRNAs in leukocytes in response to microbes, their products, and cytokines. *J. Immunol.* 168, 554–561.
Zezafoun, H., Decreux, A., and Desmecht, D. (2011). Genetic and splice variations of Bos taurus CD46 shift cell permissivity to BVDV, the bovine pestivirus. *Vet. Microbiol.* 152, 315–327.

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Immune response from a resource allocation perspective

Wendy M. Rauw*

Departamento de Mejora Genética Animal, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Madrid, Spain

Edited by:
Ilias Kyriazakis, Newcastle University, UK

Reviewed by:
Ilias Kyriazakis, Newcastle University, UK
Nicolas Friggens, Institut National de la Recherche Agronomique, France

***Correspondence:**
Wendy M. Rauw, Departamento de Mejora Genética Animal, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Crta. de la Coruña, km. 7.5, 28040 Madrid, Spain.
e-mail: rauw.wendy@inia.es

The immune system is a life history trait that can be expected to trade off against other life history traits. Whether or not a trait is considered to be a life history trait has consequences for the expectation on how it responds to natural selection and evolution; in addition, it may have consequences for the outcome of artificial selection when it is included in the breeding objective. The immune system involved in pathogen resistance comprises multiple mechanisms that define a host's defensive capacity. Immune resistance involves employing mechanisms that either prevent pathogens from invading or eliminate the pathogens when they do invade. On the other hand, tolerance involves limiting the damage that is caused by the infection. Both tolerance and resistance traits require (re)allocation of resources and carry physiological costs. Examples of trade-offs between immune function and growth, reproduction and stress response are provided in this review, in addition to consequences of selection for increased production on immune function and *vice versa*. Reaction norms are used to deal with questions of immune resistance vs. tolerance to pathogens that relate host health to infection intensity. In essence, selection for immune tolerance in livestock is a particular case of selection for animal robustness. Since breeding goals that include robustness traits are required in the implementation of more sustainable agricultural production systems, it is of interest to investigate whether immune tolerance is a robustness trait that is positively correlated with overall animal robustness. Considerably more research is needed to estimate the shapes of the cost functions of different immune strategies, and investigate trade-offs and cross-over benefits of selection for disease resistance and/or disease tolerance in livestock production.

Keywords: life history theory, resource allocation, selection, immune function, tolerance, resistance, robustness

INTRODUCTION: IMMUNE FUNCTION IS A LIFE HISTORY TRAIT

Life history theory deals with the way an organism spreads its reproduction over its lifetime and forms an adaptation to the environment it lives in (Brommer, 2000; Van Straalen and Roelofs, 2006). It is commonly defined as a set of evolved behavioral and physiological strategies that more or less influence longevity and reproduction and may include fitness traits such as reproductive success, survival, viability, fecundity, mating success, and age at maturity (Schluter et al., 1991; De Jong, 1994; Ricklefs and Wikelski, 2002). In the absence of trade-offs, natural selection would drive all life-history traits to limits imposed by animal design, where the evolutionary ideal would be an organism that matures upon birth and reproduces non-stop, producing clones of itself and never dying. However, a fundamental assumption of life history theory is that resources are limited and need to be invested amongst growth, reproduction, and maintenance, or stored for future use, and since resources used for one purpose are no longer available for other purposes, trade-offs are inevitable (Leroi, 2001; McDade, 2005; Van Straalen and Roelofs, 2006; Roff, 2007). Natural selection results in the optimal allocation of resources across important life history functions and prunes away less-optimal strategies (Brommer, 2000): "The vigorous, the healthy, and the happy survive and multiply" (Darwin, 1872).

Although the majority of life history studies focus on factors related to reproduction and growth, fitness does not only depend on reproductive success, but also on maintenance of existing structures and longevity (Lochmiller and Deerenberg, 2000). The immune system is a major physiological system centrally involved in cellular renewal and repair, and as such, it is an essential component of body maintenance (McDade, 2005). Parasites and pathogens are the greatest threat to survival by most animals, where the immune system is the major physiological mechanism regulating host survival (Lochmiller and Deerenberg, 2000). Therefore, the immune system is a life history trait which can be expected to trade off against other life history traits according to theory. These trade-offs are likely to influence not only how vigorously an organisms defends itself, but also which of the parts of the immune systems are emphasized (Lee et al., 2008).

Whether or not a trait is considered to be a life history trait has consequences for the theory on how it responds to natural selection and evolution. In addition, it may have consequences for the outcome of artificial selection when it is included in the breeding objective. According to the Resource Allocation Theory developed by Beilharz et al. (1993), when the amount of resources increases (because of a favorable environment) these resources will be used by the organism to raise fitness: "organisms respond to natural selection until fitness can improve no more. That is

the point at which organisms utilize all available resources of the environment most efficiently." Selection for high fitness by natural selection will lead to intermediate optimal values for the fitness components and heterozygosity which imparts a buffering capacity to a wide range of environments (Dunnington, 1990; Beilharz et al., 1993). However, animals that originate from a population selected for a trait requiring resources may preferentially allocate resources to this trait, reducing the availability of resource to respond to other demands. Rauw et al. (1998) and Rauw (2007) showed that the highly favorable increase in production levels in broilers, pigs and dairy cattle is often compromised by behavioral, physiological, and immunological problems. Likewise, increasing energy expenditure on maintenance related traits may reduce the availability of resources for production. When artificial selection for immune function is considered, it is of interest to evaluate the costs and possible trade-offs of immune mechanisms. This is the aim of this review.

IMMUNE FUNCTION: RESISTANCE vs. TOLERANCE

RESISTANCE

The immune system involved in pathogen resistance comprises multiple complementary, interdependent subsystems that either prevent pathogens from invading, or eliminate the pathogens when they do invade, i.e., they directly reduce the reproductive potential of the pathogen and limit the pathogen burden (Roy and Kirchner, 2000). The innate, non-specific defenses recognize antigens that are general to a wide range of pathogens and entail a series of actions that transpire almost immediately after recognition of an invading pathogen (Janeway and Medzhitov, 2002; Kogut, 2009). All multicellular organisms have some kind of innate defense; roughly 98% of all multicellular organisms possess only an innate immune system for protection against infections (Kogut, 2009). The costs of constitutive innate immunity have not been definitively measured, but the developmental costs are thought to be comparatively low because of the lack of a diversification process, low rates of cell turnover when an immune response is not being mounted, and the small tissue mass accounted for by the cells and proteins involved in the innate response (Lee, 2006). However, the constitutive components of the innate immune system can induce local inflammation via the production of inflammatory cytokines, and if highly stimulated induce the highly costly systemic inflammatory response, which is characterized by increased production of acute phase proteins by the liver, changes in energy and nutrient metabolism, anorexia and fever, leading to localized tissue damage and potentially sepsis (Cohen, 2002; Lee, 2006; Kogut, 2009).

The adaptive, specific immune defenses utilize receptors on T and B lymphocytes that recognize specific antigens on pathogens with great precision. They are characterized by an enormous range of diversity in antigen-binding receptors and have the ability to recognize and respond more quickly to antigens upon second exposure through immunological memory (McDade, 2005; Bowden et al., 2007). They are generally divided into cell-mediated and humoral components. Cell-mediated immunity (type one T-helper cells and cytotoxic T-lymphocytes) primarily defends against intracellular pathogens such as viruses, and similar to induced innate immunity, cell-mediated responses are accompanied by the secretion of proinflammatory cytokines and are sometimes associated with the nutritionally expensive systemic inflammatory response (Lee, 2006). In addition, the rapid expansion of T-cells during development and later diversification require substantial time and nutrients (Lee, 2006). An estimated 95% of maturing T cells is destroyed in the thymus as a result of rigorous selection procedures, making this a very expensive process (McDade, 2005). The costs of using the humoral component (B-cells and type two T-helper cells) are thought to be small compared with those of innate and cell-mediated defenses because the humoral immunity is associated with the production of anti-inflammatory cytokines; however, lymphocyte proliferation and diversification during the developmental period require substantial energy and nutrients (Lee, 2006). The adaptive defense is of more recent evolutionary origin and occurs in jawed vertebrates, although highly discriminatory defense responses have been identified in a number of invertebrate groups, suggesting that pathogen-specific responses might have evolved in numerous occasions and that disease-specific immunity might be commonplace in the animal kingdom (Råberg et al., 2002; Bowden et al., 2007). Immune responses mediated by T and B cells are protective to the host, but may become deleterious when immune reactions are misguided or excessive, resulting in serious damage to the host from autoimmunity or allergy (Sakaguchi et al., 2008).

Activation of the innate response is generally considered to be more costly than activation of the adaptive response (Lee et al., 2008; Colditz, 2009; Sykes, 2010). However, during re-exposure of the host to pathogens there may still be activation of innate immune pathways such that adaptive immunity may not be able to circumvent all the costs of innate immune responses (Colditz, 2009).

TOLERANCE

A second type of defense is pathogen tolerance, literally meaning "a change in sensitivity to an immune elicitor" (Ayres and Schneider, 2012). Tolerance involves limiting the damage that is caused by the infection and does not involve inhibiting pathogen growth or reproduction (Roy and Kirchner, 2000). Whereas much is known about the mechanisms involved in pathogen resistance, a systematic understanding of pathogen tolerance is limited, particularly in animals (Råberg et al., 2007; Schneider and Ayres, 2008). Tolerance is a concept that is not tied to one particular physiological mechanism (Ayres and Schneider, 2012). Schneider and Ayres (2008) consider three classes of mechanisms that can affect tolerance:

(1) Effector molecules that induce resistance mechanisms that can cause self-harm and as a result decrease tolerance. Tolerance to the damage caused by pathogens includes all of the mechanisms employed to regulate self-harm caused by aberrant immune responses (i.e., pathogen resistance mechanisms), such as autoimmunity or allergy.
(2) Signaling molecules that activate immune cells that do not cause pathology directly but may decrease tolerance through the damage induced by effectors of the activated immune

cells as well as additional pathology caused by other targets of the signaling molecules.

(3) (a) Toxic compounds produced by the host or pathogen resulting in damage to the host; (b) resistance responses that require a high level of energy expenditure leaving fewer resources available for repair of damage to other systems; (c) physiological changes induced by immune responses that are deleterious for other systems; (d) repair of tissue damage; (e) evolution of pathogen-specific solutions to infection.

In addition, interactions with mutualistic and commensal bacteria might reveal more tolerance mechanisms, including those encoded by pathogens themselves (Schneider and Ayres, 2008). Based on these classes of mechanisms, tolerance may be increased in a number of ways through damage prevention and damage repair. Firstly, by actively blocking immune detection, by lacking receptors that recognize a benign/mutualistic microbe, by keeping an immune response switched off until needed, or by (locally) reducing the activation of resistance mechanisms or selectively blocking specific signaling pathways. Secondly, by reducing self-harm resulting from the activation of resistance mechanisms, such as with having a higher affinity for pathogen-associated molecules than for self-molecules, or resulting from the elimination of self-reactive T-cell receptors and antibodies. Thirdly, by maintaining a sufficient resource intake and resource allocation, and fourthly by increasing tissue repair if pathology cannot be entirely prevented (Schneider and Ayres, 2008; Ayres and Schneider, 2012).

It is the sum of resistance and tolerance that defines a host's defensive capacity and both are genetically determined by many genes that affect different components of the immune system (Warner et al., 1987; Schneider and Ayres, 2008). The diverse immune responses are context specific and the costs will vary with the pathogen, the environment, resource availability, the developmental stage of the host, and the genotype of the host (Sandland and Minchella, 2003; Colditz, 2009).

METABOLIC COSTS OF THE IMMUNE RESPONSE

RESISTANCE

Immune defenses are energetically expensive; therefore, the rate at which organisms transform energy and nutrients can be expected to be elevated as a result of immune defense activation. Infection, trauma, and injury may result in a stereotypical response that includes loss of appetite, increased sleepiness, muscle aches, and fever. Fever, characterized by an adaptive increase in the set point for body temperature, is a complex, coordinated autonomic, neuroendocrine, and behavioral adaptive response which is used by nearly all vertebrates as part of the acute-phase reaction to immune challenge (Saper and Breder, 1994; Kluger et al., 1998). It has been associated with improved survival and shortened disease duration in non-life-threatening infections (Hasday et al., 2000). Fever is energy intensive, entailing an increased metabolic cost (Baracos et al., 1987; Nilsson, 2003). Depending on the species, fever requires a 7–15% increase in caloric energy production for each degree Celsius of increase in body temperature (Elia, 1992; Demas et al., 1997; Nilsson, 2003). In order to meet the accelerated rates of caloric expenditures associated with fever, the body must depend primarily on its stores of metabolizable energy (Beisel, 1977).

Metabolic rate in infected animals has been mostly investigated in small mammals and birds. Demas et al. (1997) showed that adult mice immunized with keyhole limpet hemocyanin (a relatively mild antigen that causes limited activation of the immune system) expended significantly more O_2 than control mice injected with saline and suggested that the energetic costs assessed in their study would be greatly increased with the use of more ecologically relevant antigentic challenges, such as bacteria or parasites. Mounting an immune response in male great tits injected with sheep red blood cells resulted in nearly 9% higher basal metabolic rates in the study of Ots et al. (2001). In addition, the animals also lost nearly 3% of their body mass subsequent to the immune challenge. In the study of Nilsson (2003), mass-specific resting metabolic rate, measured during the night when animals were inactive, was 17% higher for flea-invested marsh tit nestlings compared to control nestlings; nestlings have to depend on their innate immune system to take care of antigens. House sparrows injected with phytohaemagglutinin, a commonly used mitogen that activates the cell-mediated immune response, increased their resting metabolic rate with 29%. It was concluded that immune activity in wild passerines increases energy expenditure, which in turn may influence important life-history characteristics such as clutch size, timing of breeding or the scheduling of moult (Martin et al., 2003). Subsequent to immune challenge with nylon implant, white cabbage butterfly pupae increased their standard metabolic rate by nearly 8% compared to controls; this study was the first direct evidence indicating that activation of the immune system is energetically costly in insects (Freitak et al., 2003). According to Derting and Compton (2003), the cost of maintaining the immune system is minimal in wild white-footed mice (*Peromyscus leucopus*), but in contrast, there is a significant energetic cost of mounting an immune response.

Other immune activities related to pathogen resistance that require energy include the change in size and rate of turnover of cell and protein pools of the immune system; many components of the immune effector responses are highly proteinaceous in nature (Kyriazakis and Houdijk, 2006; Segerstrom, 2007; Colditz, 2009). Barnes et al. (2002) observed an increased fractional rate of protein synthesis of 141% in liver, 161% in plasma, and a 266% hemopexin fractional synthesis rate after injection with *Escherichia. coli* lipopolysaccharide in chickens. Some studies have attempted to quantify these costs experimentally. For example, Yewdell (2001) considered the overall protein economy of cells in relation to protein folding, ubiquitin-targeted proteasome-mediated degradation of proteins and the generation of peptide ligands for major histocompatibility complex (MHC) class I molecules, and Princiotta et al. (2003) quantified the macroeconomics of protein synthesis and degradation and the microeconomics of producing MHC class I associated peptides from viral translation products.

TOLERANCE

Protein turnover is also involved in immune tolerance in tissue replacement and repair when damage cannot be prevented during infection. For example, mastitis, an inflammatory reaction

of the mammary gland that is usually caused by a microbial infection results in tissue damage induced by either apoptosis or necrosis where both bacterial factors and host immune reactions contribute to epithelial tissue damage (Zhao and Lacasse, 2008). Larvae of several common species of parasitic nematodes migrate through, and often damage, host lungs (Hoeve et al., 2009). The wound is a site of intense metabolic activity characterized by dissolution and removal of necrotic tissue, containment and killing of pathogens, collagen and elastin synthesis and wound repair, cellular proliferation, and restoration of tissue integrity, requiring both energy and substrates (Bessey, 2004). Following injury, there is increased activity of protein, carbohydrate and fat-related metabolic pathways and of many ion pumps, and an increased blood flow to the damaged tissue (Bessey, 2004; Walsh, 2007). Increased protein turnover and accelerated muscle protein breakdown resulting in muscle wastage serves to mobilize amino acids for synthesis of new protein in wounds, for proliferation of phagocytes, macrophages, and other cellular components involved in wound healing, and for synthesis of acute-phase proteins and glucose in the liver (Bessey, 2004).

The deployment cost occurring when the immune system responds can be measured as an increase in metabolic activity because it uses up tangible parts of an organism's energy budget. However, the costs of maintenance functions in response to tissue damage are intrinsically difficult to measure and difficult to separate from other cell maintenance functions that are not part of the immune function (Schmid-Hempel, 2003). Consequently, little is known about the actual resource costs of immune tolerance. Repeated breakdown and resynthesis of proteins in cycles that use energy for no apparent net gain are costly and may appear to be energetically wasteful and futile. For example, if protein accretion would involve digestion, absorption, transport, uptake, and synthesis, net efficiency would fall in the range of 75–85%; turnover can reduce this efficiency by 15–40% (Baldwin et al., 1980). However, protein turnover provides the flux that is necessary for metabolic regulation and adaptation (Hawkins, 1991). The cost of tissue repair depends on the level of damage, as the larger the wound, the more intense the metabolic responses (Bessey, 2004).

EVOLUTION OF IMMUNE MECHANISMS

Evolution has led to a variety of defense mechanisms; however, a universally perfect defense has not evolved. Two lines of theories may explain the existence of variation in the success of defense. Firstly, pathogens or parasites usually evolve faster than their hosts where pathogens and parasites continuously track host defenses and evolve to bypass them (Jokela et al., 2000). Mechanisms employed by the pathogen that determine their virulence and mechanisms employed by the host to protect themselves result in parasite-mediated evolution of host phenotypes, resulting in an extremely complicated protection machinery (Roy and Kirchner, 2000; Freitak et al., 2003; Møller and Saino, 2004; Svensson and Råberg, 2010). As Haldane (1949) stated "the most that the average species can achieve is to dodge its minute enemies by constantly producing new genotypes" (in Duffy and Forde, 2009).

Employing resistance vs. tolerance mechanisms may have different consequences for the coevolutionary interactions between hosts and pathogens because of the differential consequences that these two mechanisms may have on the fitness of each (Møller and Saino, 2004; Svensson and Råberg, 2010). Theoretically, tolerance mechanisms, in compensating for damage, will increase pathogen fitness and therefore disease prevalence, resulting in an evolutionary advantage of carrying tolerance genes, driving them to fixation by selection. In contrast, by inhibiting infection, resistance mechanisms reduce pathogen fitness where the subsequent reduced disease prevalence will reduce the advantage of carrying resistance genes, which therefore cannot become fixed (Roy and Kirchner, 2000; Best et al., 2009). Plant studies suggest that tolerance and resistance might be mutually redundant, such that selection for tolerance in hosts should reduce selection for resistance, and vice versa (Svensson and Råberg, 2010). Indeed, in the study of Råberg et al. (2007), resistance and tolerance were negatively genetically correlated in laboratory mice infected with rodent malaria. However, Mauricio et al. (1997) suggest that both tolerance and resistance may coexist stably in populations of the plant species *Arabidopsis thaliana*, calling into question the likelihood of mutual exclusivity suggested by other authors. The latter was supported by a study of Fornoni et al. (2004), who indicated that variable costs and benefits of tolerance and resistance can result in the maintenance of intermediate levels of the two strategies. Restif and Koella (2004) showed that resistance and tolerance can be mutually exclusive, interchangeable, or complementary components of a mixed strategy of defense, depending on the shape of the costs of resistance and tolerance. They advocated that resistance and tolerance should be regarded as complementary strategies that have different effects at individual, demographic, or epidemiological scales. However, they indicate that very little is known about the actual shapes of the cost functions in natural systems (Restif and Koella, 2004).

A second theory is based on the conceptual basis of life history theory, i.e., the notion that immune systems are costly to produce, run, and maintain, and will therefore trade off against other life history traits. For example, it is hypothesized that species that develop quickly with rapid growth and short life spans invest relatively little in defenses but favor investment in growth and early reproduction, whereas species that develop slowly, with more gradual growth and longer life spans and therefore with a higher likelihood of parasite encounter, invest more resources into costly defenses (Johnson et al., 2012). Indeed host traits such as body size, development time, clutch size, lifespan, and morphology have been found to correlate with host parasitemia or immunological defenses in birds, mammals, humans, plants, and reptiles (Johnson et al., 2012). Results by Lee et al. (2008) support the hypothesis that bird species with fast life histories have immune defenses that are characterized by an emphasis on developmentally inexpensive innate constitutive defenses despite the high costs when activated (Lee, 2006). Adults of fast living species rely more heavily on rapidly developed complement proteins (a constitutive component of the innate immune system), than adults of slow-living species who utilize antibody-mediated immune defenses (a component of adaptive immune defense) more heavily. Individuals of slow living species presumably face a greater

number of infections overall and are more likely to encounter the same pathogen multiple times. Because adaptive immunity tends to have lower costs of use, high natural antibody titres may allow slow-living species to reduce the immediate costs of pathogen exposures (Lee et al., 2008).

Both tolerance and resistance traits require (re)allocation of resources and carry physiological costs (Møller et al., 1998; Roy and Kirchner, 2000), but the correlations of resistance and tolerance with other life-history traits may be different (Restif and Koella, 2004). The evolved function of an immune response is to protect an individual from harm caused by a pathogen which may be measured and defined not only immunologically, but also functionally. It is generally assumed that a strong immune response (i.e., pathogen resistance, e.g., higher antibody titres) is better than a weak immune response as animals in such a case are said to be immunosuppressed, immunocompromised, less immunocompetent or even immune-incompetent. However, achieving optimal fitness in a particular environment does not necessarily mean all fitness traits are expressed at their optimum (Allen and Little, 2011). From a cost/benefit perspective, a partially effective immune response can achieve the greatest fitness benefits. For example, where "more immunology" may result in immunopathology, the cost of eliminating or preventing the infection (resistance) may outweigh the cost of living with the infection (tolerance) (Hanssen et al., 2004; Viney et al., 2005). It is therefore conceivable that the cost to the individual of responding to infection (expenditure of metabolic resources, host-induced pathology, and compromised response to other parasite species) may favor a selective advantage of a more moderate response and tolerance (Behnke et al., 1992; Zuk and Stoehr, 2002). Or as Kogut (2009) states: "an optimal immune response to an infection might not be fully immunocompetent but would be immunosufficient or immunoresponsive."

The two theories were combined in the work of Jokela et al. (2000), who hypothesized that effectiveness of different defense mechanisms by the host is closely linked to the diversity of attack types by the enemies resulting from ongoing coevolution between hosts and enemies. In the presence of pathogens and parasites, a high diversity of attack mechanisms by the enemy inherently reduces the effectiveness of defense by the host; as effectiveness of defense decreases, the optimal allocation of resources to defense may flip from resistance to tolerance mechanisms (Jokela et al., 2000). In addition, optimal immune function is not required for survival under most circumstances such that fitness may be lowered in defended individuals in the absence of enemies (Jokela et al., 2000; Segerstrom, 2007). This is supported by a study by Sawalha et al. (2007), who showed that in sheep, PrP genotypes associated with higher susceptibility to scrapie are associated with improved postnatal survival in the absence of the disease which indicates that this susceptibility allele has selective superiority in the absence of infection. Modeling by Doeschl-Wilson et al. (2009) indicates that unfavorable associations of the scrapie resistant PrP haplotypes with post-natal lamb mortality can increase scrapie prevalence during an epidemic, and result in scrapie persisting in the population. In the study of Kraaijeveld and Godfray (2008), after 15 generations of selection for resistance to a fungal pathogen in *Drosophila melanogaster*, selected flies had lower fitness than control flies in the absence of fungal infection. If resistance depends on possessing the machinery necessary to mount a defense should infection occur, then counter-selection in the absence of the pathogen is likely in favor of tolerance mechanisms (Jokela et al., 2000; Zuk and Stoehr, 2002).

RESOURCE INTAKE

Life history patterns result from expenditure of energy and specific nutrients on fitness-related activities (Boggs, 1992; Ricklefs and Wikelski, 2002; Rauw, 2009). If the sum of energy expenditure does not match the energy intake, the balance is buffered by the storage capacity of the system. In the long-run, however, energy intake must balance energy expenditure (Weiner, 1992). Infection results in the disruption of normal processes of nutrient intake, digestion, and absorption (Lochmiller and Deerenberg, 2000). The nutritional responses during a generalized infection include alterations in rates of protein synthesis and degradation, fatty acid and carbohydrate metabolism, and alterations in the metabolic processing of individual amino acids, electrolytes, minerals, trace elements, and vitamins (Beisel, 1977). There is a particular emphasis on the ability of host tissues to manufacture specific key proteins in sufficient quantity since both the immune response (pathogen resistance, including lymphocyte proliferation, antibody production, and cytokine release) as well as the repair of cellular and tissue damage (pathogen tolerance) are all dependent upon protein-synthesizing mechanisms (Beisel, 1977; Lochmiller and Deerenberg, 2000). Certain types of proteins are synthesized at accelerated rates, whereas many individual amino acids may be wasted to accelerated processes of, for example, gluconeogenesis (Beisel, 1977; Le Floc'h et al., 2004). The acceleration of protein catabolism results in protein malnutrition and wasting of body tissue; protein malnutrition is instilled in a few days while this would take several weeks to develop during simple starvation (Beisel, 1977; Lochmiller and Deerenberg, 2000; McDade, 2005).

The sharply negative body nitrogen balance is exacerbated by a marked reduction in dietary food intake during the period of acute illness, although nitrogen may be lost from the body without the absence of a diminished dietary intake (Beisel, 1977). One of the earliest responses to infection is cytokine-mediated anorexia, where interleukins 1, 6, and 8, tumor necrosis factor and interferon alpha are released by the host defense mechanisms resulting in reduced nutrient intake through effects on the central nervous system (Donabedian, 2006). The immune system does not have to be challenged to a great degree to alter nutrient dynamics in the host because even rather mild immune reactions, like those associated with vaccination, can suppress feed intake and development (Lochmiller and Deerenberg, 2000). Because infection-induced reduction in food intake seems paradoxical during a period of high energy expenditure, traditionally, anorexia was thought of as an adverse secondary response to infection that served no function to the host. However, since this response is common among animals, it is now hypothesized that anorexia might rather be an adaptive trait that modulates the host's ability to fight infection (Ayres and Schneider, 2009). Kyriazakis et al. (1998) proposed that anorexia during parasitic

infection is an evolved adaptation of the host for promoting an effective immune response and for becoming more selective in its diet toward foods that either minimize the risk of infection or are high in antiparasitic compounds. Anorexia means that demands for amino acids to support immune activation must be met from the proteins stored in the body. However, the amino acid pattern required to support immune response is different from that released by skeletal muscle proteolysis, resulting in an excess of non-limiting amino acids, whereas others become limiting for immune response. This internal amino acid balance in which the supply of muscle protein does not match the demand results in tissue loss and eventually malnutrition (Reeds and Jahoor, 2001; Le Floc'h et al., 2004).

The influence of malnutrition on resistance to infection is well established since it is the primary cause of immunodeficiency in humans worldwide (e.g., Tomkins, 1986; Katona and Katona-Apte, 2008; Panda et al., 2010). Several studies, but mostly in ruminants, have investigated the influence of nutrition and dietary manipulation on the ability of the host to cope once infected. According to Coop and Sykes (2002), evidence in the literature supports the view that protein supplementation has little or no effect on the ability of young growing livestock to prevent the early establishment of a parasite infection, however, the major effect of protein appears to be on the speed or degree to which the animal can express immunity against an established parasitic challenge. Van Houtert et al. (1995) and Butter et al. (2000) observed that worm egg concentrations in faeces were significantly reduced and apparent rate of worm expulsion considerably increased when sheep where given protein supplementation while infected with *Trichostrongylus colubriformis*. Likewise, dietary crude protein content decreased faecal worm egg counts significantly after infection with Haemonchus contortus in the study of Datta et al. (1998). The literature review by Knox and Steel (1996) concluded that low cost supplements, which supply nitrogen and essential minerals, will reduce the effects of parasitic infection in small ruminants by increasing weight gain and wool production and reducing faecal egg output and parasite burden. Sykes and Coop (2001) state that both resistance of sheep to larval establishment and performance during larval challenge can be enhanced by improved protein nutrition. In addition to protein, several other nutrients are known to influence immune functions, including vitamins, minerals, and fatty acids, therefore, in theory, scarcity of any of these nutrients may cause reduced resistance to infection to some extend (Houdijk et al., 2001).

Kyriazakis et al. (1994) observed that sheep infected daily with a small number of larvae of the small-intestinal parasite *T. colubriformis* are actually able to choose a diet high in protein content in order to meet the increased protein requirements resulting from such an infection. Similar results were found in larvae of caterpillars *(Spodoptera littoralis)* experimentally challenged with a highly virulent entomopathogen (nucleopolyhedrovirus) in the study of Lee et al. (2006) and in larvae of the African armyworm *(Spodoptera exempta)* experimentally infected with an opportunist bacterium *(Bacillus subtilis)* in the study of Povey et al. (2009). Both studies showed that infected larvae selected diets with higher levels of protein relative to uninfected larvae when offered a higher protein diet choice. In the widest sense, successful diet selection can be described as self-medication, with animals choosing a greater or lesser proportion of a food in order to match its optimum intake to defend itself against an illness (Forbes, 2007). Specific amino acid requirements need to be taken into account in order to preserve muscle mass and performance of farm animals (Le Floc'h et al., 2004).

RESOURCE ALLOCATION AND TRADE OFFS

RESOURCE PRIORITY AND HOMEORHESIS

Organisms can be thought of as being informed resource users which have evolved diverse resource management systems to cope with a variety of challenging environmental conditions (Glazier, 2009a). Because of limited and variable availability of resources, organisms have evolved priority systems for allocating resources to various activities and structures in a hierarchical fashion (Glazier, 2009b). Some organ systems, such as the brain and the heart, have high energetic priority at all times, whereas others, including the immune system, can be spared when necessary (Segerstrom, 2007). In addition, there may be good adaptive reasons for not overlapping different life-cycle stages, such that control mechanisms may constrain certain combinations of physiological, behavioral and anatomical states from occurring together (Ricklefs and Wikelski, 2002). There is abundant evidence indicating that at different stages of the life cycle various metabolic pathways are up- or down-regulated resulting in nutrients that are divided in various amounts to different tissues, biological functions and end products (Collier et al., 2009). This change in tissue responses to homeostatic controls is called homeorhesis, which represents "the orchestrated or coordinated changes in metabolism of body tissues to support a physiological state" (Bauman and Currie, 1980; Collier et al., 2009). Homeorhesis was initially extensively described for the physiological state of lactation where marked alterations in the partitioning of nutrients and metabolism of the animal occur to accommodate the demands of the mammary gland. In addition, the preference of other body tissues for nutrients is altered to allow partitioning of a greater percentage of glucose to the mammary gland (Bauman and Currie, 1980). Meanwhile, the general concept of homeorhesis has been extended to include many other physiological states, nutritional and environmental conditions and pathological states as summarized in Collier et al. (2005). Also infection elicits a complete shift in metabolic priorities within the host to those associated with immunity (Lochmiller and Deerenberg, 2000; Le Floc'h et al., 2004). Spurlock (1997) discussed the physiological processes that take place during periods of immune challenge, in which pro-inflammatory cytokines orchestrate a homeorhetic response directing nutrients away from tissue growth in support of immune function. This cytokine-mediated "reprogramming" of nutrient uptake and utilization ensures an adequate supply of nutrients for proliferation of lymphocyte and macrophage populations, antibody production, and hepatic synthesis of acute phase proteins (Spurlock, 1997). A study by DiAngelo et al. (2009) in *Drosophila melanogaster* suggested that activation of the Toll signaling pathway in fat suppresses insulin signaling, leading to a decrease in nutrient stores

and growth. The authors suggest that communication between these regulatory systems evolved as a means to divert energy in times of need from organismal growth to the acute requirement of combating infection.

Maintenance (survival or longevity) is usually given precedence over growth and reproduction when animals are given limited food, or are stressed in other ways as this will guarantee survival in the short term (Kyriazakis and Houdijk, 2006; Glazier, 2009a). For this reason, maintenance functions are relatively insensitive to (moderate) changes in nutrient supply. Traditionally, immune functions have been regarded as part of maintenance; however, evidence shows that at least some aspects of immunity are sensitive to changes in nutrient intake (Coop and Kyriazakis, 1999). When resources are limited, in some situations it could be adaptive for organisms to direct energy away from the immune system toward protecting and restoring other functions which may manifest itself in the form of tradeoffs (Segerstrom, 2007).

McNamara and Buchanan (2005) hypothesize that under stressful conditions animals must allocate their limited resources between the competing demands of combating the stressor (resistance) and maintaining condition (tolerance). Increasing allocation of resources to combating the stressor will leave fewer resources for adequate maintenance, increasing the chance of mortality due to the build-up of damage. This is also suggested by Segerstrom (2007) who hypothesized that energy used by the immune system represents a lost opportunity to spend that energy remediating resource loss and resolve other demands. According to the model by McNamara and Buchanan (2005), in a situation of resource restriction, the optimum strategy for resource allocation to combating an immediate physiological threat depends on the cost to individual condition and the threat and duration of the stress period. The optimal strategy concerning the immune system will depend on the pathogenicity of the environment as well as on the body condition and the costs and success of mounting an immune response (Lochmiller and Deerenberg, 2000).

Speakman (2008) suggested that the reduced immunocompetence observed during lactation may not be a compensatory cost resulting from diverting resources away from immunocompetence toward lactation, but a consequential cost resulting from a reduction in fat content and subsequent changes in circulating levels of leptin. Leptin directly influences immune cells, stimulating T-cell immunity, phagocytosis, cytokine production and haemopoiesis, resulting in attenuated susceptibility to infection. French et al. (2007) termed this the "obligate regulation hypothesis," where immune function will be suppressed in all reproductive animals regardless of energetic state because of circulating hormone concentrations. For example, the action of sex steroids may influence both reproduction and immune function. However, since food availability does have a profound effect on immune function, they rejected this hypothesis and supported instead the "facultative regulation hypothesis" which states that energy resource availability is the driving force behind the context-dependent relationship between reproductive and immune systems, with functional trade-offs only occurring when resources are limited.

Discrepancies between studies investigating trade-offs may be a result of differing resource availability because energy conflicts may only manifest during resource-intensive times (French et al., 2007). This is supported by work of Doeschl-Wilson et al. (2009), who showed in a mathematical model that the relationship between a host's response to pathogen challenge and production potential largely depends on the interaction between its genetic capacity for production and disease resistance with the nutritional environment. The observation that selection for high production efficiency has resulted in several undesirable side effects that are mostly related to metabolic imbalance, i.e., a mismatch between increased output (selection for high production) and decreased input (selection for increased feed efficiency and reduced body fat reserves), suggests that we can expect our farm animals to be restricted by their environment (Rauw, 2009). Trade-offs may not be found at all if two processes do not share important resources, if resources are not limited or if the trade-off does not involve the immune parameter being measured (Lee, 2006). In addition, several parts of the defense mechanisms may not incur significant fitness costs (Coustau et al., 2000; Rigby et al., 2002).

TRADE-OFF BETWEEN IMMUNE FUNCTION AND GROWTH

The negative influence of activation of the immune system on growth is well established resulting both from a reduced feed intake through anorexia and from redirection of resources toward an immune response away from other functions. For example, chronic immune stimulation in non-vaccinated sows that were farrowed in a non-sanitized farrowing room and that did not receive antibiotics resulted in reduced body weight gains in pigs in the study of Williams et al. (1997). Immune challenge with *E. coli* lipopolysaccharide resulted in reduced weight gain in weanling pigs in the study of Van Heugten and Spears (1997). Mauck et al. (2005) observed an inverse relationship between growth rate and the development of components of the avian immune system in a wild population of Leach's Storm-Petrels (*Oceanodroma leucorhoa*), although this trade-off was suggested to be more complex than resulting from simple energy allocation. Daily injections of the inflammatory agent Sephadex resulted in significantly lower rates of weight gain in chicks in the study of Klasing et al. (1987). Reciprocally, in the study of Allen and Little (2011), stimulating an increased development rate in juvenile *Daphnia* resulted in an increased infection rate when exposed to the parasite *Pasteuria ramosa*, suggesting that allocation of resources to development left the fish lacking in ability to allocate an adequate amount to parasite resistance. Coop and Kyriazakis (1999) theorized that growing animals that encounter parasites for the first time can be expected to prioritize resources to the acquisition of immunity over growth, whereas once immunity has been acquired, growth and reproduction would be prioritized over expression of immunity to parasites. Indeed, a large body of evidence shows that increased metabolizable protein supply reduces fecal egg counts and worm burdens in ruminants only at later stages in experimental parasitic infestations, which supports this view that acquisition, but not expression, of immunity takes priority over growth (Houdijk and Athanasiadou, 2003).

TRADE-OFF BETWEEN IMMUNE FUNCTION AND LACTATION

Reproductive effort, and in particular lactation, is a resource-prioritized process that requires substantial energy and other nutrient resources. The prevalence and intensity of parasitic infection often increases in animals when they are reproducing, which may result from adaptive reallocation of resources in times of increased energetic demand (Deerenberg et al., 1997). Increased brood size resulted in a reduced probability of detecting any immune response against sheep red blood cells in zebra finches (*Poephila guttata*) in the study of Deerenberg et al. (1997). Verhulst et al. (2005) suggest that zebra finches *(Taeniopygia guttata)* rearing large broods have lower antibody responses because they economize on the maintenance costs of the immune system. Furthermore, in collared flycatchers (*Ficedula albicollis*), increased brood size resulted in reduced antibody production when immunized with Newcastle disease virus in the study of Nordling et al. (1998), and in reduced T-cell-mediated immune response when injected with phytohemagglutinin in the study of Moreno et al. (1999). Breeding grey partridges (Perdix perdix) immune challenged with Newcastle disease virus laid smaller eggs, suggesting that immune challenge can have physiological consequences in terms of self-maintenance and reproductive allocation to the egg (Cucco et al., 2010).

In several species of mammals, an increasing number of experimental studies indicate that competition for nutrients between the immune system and reproductive effort may result in a peri-parturient breakdown of acquired immunity to parasites (Houdijk et al., 2001). Lactating ewes show an increased fecundity of parasites present and an inhibition of the expulsion of established parasites, while prevention or premature termination of lactation results in the expulsion of established parasites and rejection of newly acquired infection (Shubber et al., 1981). Lactating bighorn ewes had greater faecal counts of lungworm larvae compared with non-lactating females, suggesting that reproduction resulted in a decrease in resistance to parasites and pathogens (Festa-Bianchet, 1989). Ewes that have acquired immunity to nematode infection tend to lose it around the time of parturition and during lactation, and strains of sheep selected for resistance to nematode infection still undergo a peri-parturient loss of immunity (Barger, 1993). However, Xu et al. (2012) showed that immune function is not suppressed to compensate the high energy demands during lactation in Brandt's voles.

TRADE-OFF BETWEEN IMMUNE FUNCTION AND STRESS RESPONSE

Trade-offs may result from resources that are allocated to deal with external stresses (Svensson et al., 1998). The stress response includes metabolic, energetic, immune, endocrine, neural, and behavioral changes that are aimed at overcoming the stressful situation and compensating for the imbalances produced by the stressor (Selye, 1953; Tort, 2011). Stress, through the action of stress hormones such as glucocorticoids, catecholamines, prolactin, growth hormone and nerve growth factor, has detrimental effects on immune function (Moberg, 2000; Webster Marketon and Glaser, 2008). Cortisol simultaneously makes more glucose available from energy stores and suppresses certain physiological activities such as immune activity and reproduction (Segerstrom, 2007). In addition, the consequences of stress include elevated metabolic costs since energy is needed by the animal to cope with the stress.

The stress model developed by Moberg (2000) explains the concept of trade-offs between stress and other functions. An animal has a budget of resources that are available to service basal biological functions, in addition, the animal has available a reserve from which it must draw to deal with stress. The biological cost of stress depends on the duration of the stress (acute vs. chronic), the severity of the stressor, and on the number of stressors (or repeated exposure to the same stressor). When the biological cost is met by the reserves, the stressor will have no impact on the other biological functions; however, when there are insufficient biological reserves available, resources must be reallocated away from other biological functions that now become impaired. At this time the animal enters a pre-pathological-pathological state due to a reduction in its physiological state, and experiences distress (Moberg, 2000).

Environmental stressors are involved in the aetiology of important livestock diseases, including transmissible gastroenteritis in young pigs, Newcastle and Marek's disease in chickens and shipping fever in cattle (Kelley, 1980). In an extensive review, Kelley (1980) identified eight stressors that typically occur in modern livestock production systems: heat, cold, crowding, mixing, weaning, limit-feeding, noise, and restraint and all of these stressors have been shown to alter the immune system of animals. Effects of stress on immune function in fish have been reviewed by Tort (2011). When the stressor is acute and short-term, the response pattern is stimulatory and the fish immune response shows an activating phase that specifically enhances innate responses; however, if the stressor is chronic, the immune response shows suppressive effects and therefore the chances of an infection may be enhanced (Tort, 2011). In humans, acute stressors enhance low-energy-consuming immune components and suppress high-energy-consuming ones, whereas stressors lasting from days to years are associated with suppression of a number of different immune functions, including protein production, cell production, and cell function (Segerstrom, 2007). Strenuous stress also tends to suppress several aspects of immune function and, vice versa, costly behaviors are reduced in animals mounting an immune response (Svensson et al., 1998; Viney et al., 2005). Sickness behavior that is characterized by increased fatigue, sleep, withdrawal and a decreased interest in pleasurable behaviors is initiated by the host as a result of activation of the immune system (Segerstrom, 2007).

CONSEQUENCES OF SELECTION FOR INCREASED PRODUCTION ON IMMUNE FUNCTION AND VICE VERSA

Genetic selection has increased production levels of livestock species considerably; however, animals in a population that have been selected for high production efficiency appear to be more at risk for behavioral, physiological, and immunological problems (Rauw et al., 1998). Artificial selection may result in preferential allocation of resources to the traits selected for, leaving animals lacking in ability to respond adequately to other demands. In particular those traits that are not specifically included in the

breeding goal may be affected, i.e., traits other than production traits, because their importance is not specifically recognized (Rauw, 2009).

Genetic selection of poultry for superior growth rate may result in decreased resistance to disease or reduced immunological response (Bayyari et al., 1997). A meta-analysis by Van der Most et al. (2011) indicated that selection for accelerated growth in poultry had a large and significantly negative effect on immune function. Chickens from a line selected for faster growth were more susceptible to the development of Marek's disease than chickens from a line exhibiting a slower growth rate in the study of Han and Smyth (1972). Broilers selected for high growth rate showed lower antibody responses when challenged with sheep erythrocytes (SRBC) than animals from a low body weight line (Miller et al., 1992) and a randombred control line (Qureshi and Havenstein, 1994). Koenen et al. (2002) conclude that fast growing broiler chickens are specialized in the production of a strong short-term humoral response, whereas slow growing layer-type chickens are specialized in a long-term humoral response in combination with a strong cellular response, which is in conformity with their life expectancy. In the study of Saif et al. (1984), a natural outbreak of erysipelas and fowl cholera resulted in a higher mortality rate in turkeys from a line selected for increased growth rate than in animals from an unselected control line. Mortality of turkeys from the selected line was higher than that of animals from the unselected control line when subsequently experimentally challenged with *Pasteurella multocida* (Sacco et al., 1991; Nestor et al., 1996a,b) or with Newcastle disease virus (Tsai et al., 1992). In addition, animals from the fast growth line had a lower toe web response to phytohemagglutinin-P, lower lymphocyte counts, and lower relative spleen weights than animals from the randombred control line (Bayyari et al., 1997). In mice, Coltherd et al. (2009) concluded that artificial selection for high growth may reduce the ability to cope with pathogens and that improved protein nutrition may to some extent ameliorate this penalty.

In dairy cattle, overall, there is clear evidence that there are negative genetic associations between milk yield and health (Veerkamp et al., 2009). Clinical mastitis cases are principally associated with one of the following bacteria: *S. Aureus, E. Coli, Streptococcus dysgalactiae, Streptococcus uberis, CNS, Arcanobacterium pyogenes,* or *Klebsiella spp* (Rupp and Foucras, 2010). The genetic antagonism between milk yield and mastitis resistance has been well established (Rupp and Boichard, 2003). The average genetic correlation between milk yield and mastitis was reviewed to be 0.30 across seven studies by Emanuelsson (1988), 0.38 across 16 studies by Pryce et al. (1997), and 0.43 in Nordic data by Heringstad et al. (2000). After four generations of selection for milk production in a divergent selection experiment in dairy cattle, the genetic difference in mastitis between the high and low milk production group was 3.1% clinical mastitis as a correlated response (Heringstad et al., 2003). Although studies are rare for goats and sheep, they do confirm the positive relationship between milk yield and measurements of mastitis (Raynal-Ljutovac et al., 2007). Rupp and Boichard (2003) suggest that pleiotropic genes could be involved, but also biological competition for energy and nutrients between functions.

Romney sheep selected for increased fleece weight had higher feacal worm egg counts (Howse et al., 1992); Eady et al. (1996) estimated that genetic selection for productivity in sheep would lead to a 1% per annum increase in feacal worm egg counts. In Australia and New Zealand, egg counts following nematode infection are unfavorably correlated with wool growth and live-weight; however, these correlations are consistently favorable in Europe (Stear et al., 2001).

Reciprocally, divergent selection for sheep red blood cell antibody response in a White Leghorn population resulted in reduced body weight in the studies of Gross et al. (2002) and Lamont et al. (2003). Martin et al. (1990) observed that females from the low line were heavier as juveniles but lighter as adult, matured at a younger age, and had higher egg production than those from the high line. In the study of Lamont et al. (2003), a difference in body weight was observed as early as 7 days after hatch; after 20 generations of selection, animals from the line selected for high antibody response were 20% lighter and matured 30 days later than animals from the line selected for low antibody response. Selection for resistance to Marek's disease in chickens resulted in animals with lower adult body weight and smaller eggs than animals from unselected lines (Warner et al., 1987).

Selection for reduced helminth feacal egg counts may result in lower lamb growth rates (Bisset et al., 2001). In the study of Morris et al. (2000), selection for low feacal worm egg count in Romney sheep resulted in decreased post-weaning weight gain and decreased fleece weight in yearlings and ewes. Tendencies toward unfavorable relationships between immune-competence and lean growth capacity have been reported in growing pigs (Knap and Bishop, 1996). The genetic trend for protein yield after four generations of selection for milk production in a divergent selection experiment in dairy cattle was significantly negative in a line selected for low clinical mastitis, corresponding to −1.97 kg protein per cow per generation (Heringstad et al., 2003).

TRADE-OFFS WITH IMMUNE TOLERANCE

The trade-offs described above between production traits and immune function may be mostly ascribed to immune resistance, although immune tolerance mechanisms such as damage repair may have been involved. Trade-offs with immune tolerance seems to be difficult to consider because of the difficulty in separating the processes involved in damage repair from other cell maintenance functions, in addition, literature on immune tolerance in animals is scarce. Immune tolerance is correctly evaluated by measuring the fitness response to a gradient in intensity of infection (Simms, 2000), and such data is not yet available. Trade-offs between protein turnover and production traits have been described in non-immune challenged animals. For example, selection for increased growth rate has resulted in slower protein turnover rates and reduced energy requirements for maintenance in rats (Bates and Millward, 1981), chickens (Thomas et al., 1991), lambs (Oddy et al., 1995) and cattle (Richardson and Herd, 2004). Increasing the degree and/or effectiveness of cell and tissue maintenance functions with selection for immune tolerance can be expected to result in higher energy and protein expenditures and consequently trade-offs with other economically important production traits.

However, improving tolerance mechanisms may have positive consequences for overall adaptability and robustness. For example, protein turnover provides the flux that is necessary for metabolic regulation and adaptation. It enables the metabolic adjustments required for maintenance of homeothermy, reproduction, and development, the repair of damaged tissue, maintenance of the immune system in a state of readiness, combating infection, and during or following changes in the environment or in the nutritional/physiological status (Hawkins, 1991; Lobley, 2003). Pirlet and Arthur-Goettig (1999) suggest that the evolution of life results from specific degradation of defective, old, damaged, denatured protein molecules, which forces the selection of structurally superior proteins. Protein turnover is furthermore involved in the ageing process, in the maintenance and error correction of functional proteins through the removal of proteins damaged by oxidative stress (Tavernarakis and Driscoll, 2002). Hawkins (1991), in an excellent review, indicates that intense whole-body protein turnover may enhance viability by enabling the metabolic adjustments necessary for regulation and adaptation. Faster protein turnover may enhance performance by improvement of sensitivity in metabolic and endocrine control, facilitating faster acclimation in the regulation of metabolic flux, as well as functioning in the mobilization and selective redistribution or catabolism of amino acids, elimination of non-functional or denatured polypeptides, and thermogenesis. Thus, improved protein turnover rate may improve the ability of an animal to adapt to new dietary and physiological conditions in addition to immune tolerance, i.e., improve robustness (Baldwin et al., 1980).

Phenotypic changes across environments for a wide variety of different characters in plants and animals, in natural and agricultural systems, and over both temporal and spatial variation in the environment is the basis of "phenotypic plasticity" which is determined by the shape of the reaction norm of the phenotypic values expressed by a genotype across a range of environments (Via et al., 1995). Plant ecologists have adapted the method to deal with questions of resistance vs. tolerance to pathogens with reaction norms that relate host health to infection intensity. Resistance is a measure of the ability of a host to limit pathogen growth and thereby maintain health, which can be interpolated as the inverse of the mean of the pathogen load. Tolerance is a measure of the ability of a host to survive an infection at a given pathogen load, which is represented by the slope of the curve (Simms, 2000; Schneider and Ayres, 2008). Thus, improving resistance would consist of moving the animal up the reaction-norm curve toward a lower pathogen load and higher health, whereas improving tolerance would entail flattening the slope of the curve. Råberg et al. (2007) conclude that this method is readily transferable to domestic animals where it could be used to work out optimal selection strategies to enhance immune defense mechanisms.

A tolerant genotype minimizes the decline in fitness from that achieved in a relatively benign environment to that in a relatively stressful environment; thus, measuring tolerance involves measuring fitness in more than one environment (Simms, 2000). In essence, selection for immune tolerance in farm animal species is a particular case of selection for animal robustness. Robustness is defined by Knap (2005) as "the ability to combine a high production potential with resilience to stressors, allowing for unproblematic expression of a high production potential in a wide variety of environmental conditions." Two options for breeding for animal robustness are extensively described by Knap (2009): the direct approach involves the inclusion of directly measurable robustness traits in the breeding objective and in the selection index, whereas an indirect approach involves the use of reaction norms analysis to estimate breeding values for the environmental sensitivity of the genetic potential for production performance. Reaction norms are a measurement of the phenotypes for a given genotype across a range of environments that measure how an individual responds to a range of environmental conditions (Schneider and Ayres, 2008). In animal production this means that progeny of sires are spread across a wide environmental range and are recorded for the production traits of interest. The production performance is then regressed on a descriptor of the environment (from a worse to a better environment, production is expected to increase) where animals with high resilience to external stressors (i.e., animals with a flatter slope) will be more robust and hence more desirable (Friggens and Van der Waaij, 2009; Knap, 2009). Because of the increasingly wide variety of environmental conditions in which livestock animals are required to perform, and evidence that expression of high production potential is more compromised in high producing animals, robustness has a high priority in current livestock production. As Mormède et al. (2010) state: the farm animal of the future is robust, adapted and healthy. Therefore, a possible relationship between immune tolerance mechanisms and other robustness traits would be highly desirable.

CONCLUSIONS: SELECTION FOR RESISTANCE OR TOLERANCE?

Breeding for immune defenses is needed to improve sustainability of livestock systems and is becoming more common throughout the world (Stear et al., 2001; Bishop et al., 2002). The distinction between resistance and tolerance is of importance since it determines the suitability of selection for different disease scenarios (Bishop et al., 2002). Both are genetically determined by many genes that indicate that selective breeding is feasible; however, both resistance and tolerance are life history traits that require (re)allocation of resources and carry physiological costs which may trade off against other economically important traits when resources are limited.

Stear et al. (2001) raise several concerns about the desirability of breeding for disease resistance. One concern is that there may be unfavorable consequences for other diseases; for example, when selective breeding for resistance to a specific disease may predispose hosts to prefer one class of immune response, leaving them susceptible to infectious agents that are normally controlled by another type of response. A counter argument is that selective breeding for resistance to immunosuppressive diseases would reduce the prevalence of these diseases and enhance overall immune responsiveness (Stear et al., 2001). In addition, it may be possible to select for resistance to several diseases by selecting for enhanced immune responsiveness (Wilkie and Mallard, 1999; Stear et al., 2001).

As outlined in the previous section, mechanisms involved in disease tolerance (damage repair) appear to be of a more general nature. In addition, mechanisms of cell maintenance and repair may be involved in adaptability to new nutritional, physiological and environmental conditions, i.e., animal robustness. Selection for increased production efficiency has narrowed the amount of resources that are available to the demands of maintenance, growth and reproduction. This reduction in metabolic space may reduce an animal's resilience to stressors and its ability to adapt to a wide variety of environmental conditions. Therefore, breeding goals that include robustness traits are required in the implementation of more sustainable agricultural production systems (Knap, 2009; Rauw, 2012). They combine robustness traits with production traits, balancing production potential with environmental sensitivity; this will increase or restore the animals' ability to interact successfully with the environment and improve welfare and productivity (Knap, 2009). It will be therefore of great interest to investigate the theory that immune tolerance is a robustness trait that may be positively correlated with overall animal robustness.

Considerably more research is needed to estimate the shapes of the cost functions of different immune strategies, and investigate trade-offs and cross-over benefits of selection for disease resistance and/or disease tolerance in livestock production.

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REFERENCES

Allen, D. E., and Little, T. J. (2011). Identifying energy constraints to parasite resistance. *J. Evol. Biol.* 24, 224–229.

Ayres, J. S., and Schneider, D. S. (2009). The role of anorexia in resistance and tolerance to infections in *Drosophila*. *PLoS Biol.* 7:e1000150. doi: 10.1371/journal.pbio.1000150

Ayres, J. S., and Schneider, D. S. (2012). Tolerance of infections. *Annu. Rev. Immunol.* 30, 271–294.

Baldwin, R. L., Smith, N. E., Taylor, J., and Sharp, M. (1980). Manipulating metabolic parameters to improve growth rate and milk secretion. *J. Anim. Sci.* 51, 1416–1428.

Baracos, E., Whitmore, W. T., and Gale, R. (1987). The metabolic cost of fever. *Can. J. Physiol. Pharmacol.* 65, 1248–1254.

Barger, I. A. (1993). Influence of sex and reproductive status on susceptibility of ruminants to nematode parasitism. *Int. J. Parasitol.* 23, 463–469.

Barnes, D. M., Song, Z., Klasing, K. C., and Bottje, W. (2002). Protein metabolism during an acute phase response in chickens. *Amino Acids* 22, 15–26.

Bates, P. C., and Millward, D. J. (1981). Characteristics of skeletal muscle growth and protein turnover in a fast-growing rat strain. *Br. J. Nutr.* 46, 7–13.

Bauman, D. E., and Currie, W. B. (1980). Partitioning of nutrients during lactation: a review of mechanisms involving homeostasis and homeorhesis. *J. Dairy Sci.* 63, 1514–1529.

Bayyari, G. R., Huff, W. E., Rath, N. C., Balog, J. M., Newberry, L. A., Villines, J. D., et al. (1997). Effect of the genetic selection of turkeys for increased body weight and egg production on immune and physiological responses. *Poult. Sci.* 76, 289–296.

Behnke, J. M., Bernard, C. J., and Wakelin, D. (1992). Understanding chronic nematode infections: evolutionary considerations, current hypotheses and the way forward. *Int. J. Parasitol.* 22, 861–907.

Beilharz, R. G., Luxford, B. G., and Wilkinson, J. L. (1993). Quantitative genetics and evolution: is our understanding of genetics sufficient to explain evolution? *J. Anim. Breed. Genet.* 110, 161–170.

Beisel, W. R. (1977). Magnitude of the host nutritional responses to infection. *Am. J. Clin. Nutr.* 30, 1236–1247.

Bessey, P. Q. (2004). "Metabolic response to critical illness," in *ASC Surgery: Principles and Practice*, 1–27. Available online at: http://129.49.170.167/Volumes/ACSCD+July+2010/ACSCD/pdf/ACS0825.pdf

Best, A., White, A., and Boots, M. (2009). Resistance is futile but tolerance can explain why parasites do not always castrate their hosts. *Evolution* 64, 348–357.

Bishop, S. C., Chesnais, J., and Stear, M. J. (2002). "Breeding for disease resistance: issues and opportunities," in *Proceedings of the 7th WCGALP* (Montpellier, France), 1–8.

Bisset, S. A., Morris, C. A., McEwan, J. C., and Vlassoff, A. (2001). Breeding sheep in New Zealand that are less reliant on helminthics to maintain health and productivity. *N.Z. Vet. J.* 49, 236–246.

Boggs, C. L. (1992). Resource allocation: exploring connections between foraging and life history. *Funct. Ecol.* 6, 508–518.

Bowden, L., Dheilly, N. M., Raftos, D. A., and Nair, S. V. (2007). New immune systems: pathogen-specific host defence, life history strategies and hypervariable immune-response genes of invertebrates. *ISJ* 4, 127–136.

Brommer, J. E. (2000). The evolution of fitness in life-history theory. *Biol. Rev.* 75, 377–404.

Butter, N. L., Dawson, J. M., Wakelin, D., and Buttery, P. J. (2000). Effect of dietary tannin and protein concentration on nematode infection (*Trichostrongylus colubriformis*) in lambs. *J. Agr. Sci.* 134, 89–99.

Cohen, J. (2002). The immunopathogenesis of sepsis. *Nature* 420, 885–891.

Colditz, I. G. (2009). "Allocation of resources to immune responses," in *Resource Allocation Theory Applied to Farm Animal Production*, ed W. M. Rauw (Wallingford, UK: CABI Publishing), 192–209.

Collier, R. J., Baumgard, L. H., Lock, A. L., and Bauman, D. E. (2005). "Physiological limitations, nutrient partitioning," in *Yields of Farmed Species: Constraints and Opportunities in the 21st Century*, eds J. Wiseman and R. Sylvestor (Nottingham, UK: Nottingham University Press), 351–378.

Collier, R. J., Limesand, S. W., Rhoads, M. L., Rhoads, R. P., and Baumgard, L. H. (2009). "Homeorhesis during heat stress," in *Resource Allocation Theory Applied to Farm Animal Production*, ed W. M. Rauw (Wallingford, UK: CABI Publishing), 72–88.

Coltherd, J. C., Bünger, L., Kyriazakis, I., and Houdijk, J. G. M. (2009). Genetic growth potential interacts with nutrition on the ability of mice to cope with *Heligmosomoides bakeri* infection. *Parasitology* 136, 1043–1055.

Coop, R. L., and Kyriazakis, I. (1999). Nutrition-parasite interaction. *Vet. Parasitol.* 84, 187–204.

Coop, R. L., and Sykes, A. R. (2002). "Interaction between gastrointestinal parasites and nutrients," in *Sheep Nutrition*, eds M. Freer and H. Dove (Wallingford, UK: CABI Publishing), 387.

Coustau, C., Chevillon, C., and French-Constant, R. F. (2000). Resistance to xenobiotics and parasites: can we count the cost? *Trends Ecol. Evol.* 15, 378–383.

Cucco, M., Pellegrino, I., and Malacarne, G. (2010). Immune challenge affects female condition and egg size in the grey partridge. *J. Exp. Zool. A Ecol. Genet. Physiol.* 313, 597–604.

Darwin, C. (1872). *On the Origin of Species by Natural Selection, or the Preservation of Favoured Races in the Struggle for Life*. London, UK: John Murray.

Datta, F. U., Nolana, J. V., Rowe, J. B., and Gray, G. D. (1998). Protein supplementation improves the performance of parasitized sheep fed a straw-based diet. *Int. J. Parasitol.* 28, 1269–1278.

Deerenberg, C., Apanius, V., Daan, S., and Bos, N. (1997). Reproductive effort decreases antibody responsiveness. *Proc. R. Soc. Lond. B* 264, 1021–1029.

De Jong, G. (1994). The fitness of fitness concepts and the description of natural selection. *Quart. Rev. Biol.* 69, 3–29.

Demas, G. E., Chefer, V., Talan, M. I., and Nelson, R. J. (1997). Metabolic costs of mounting an antigen-stimulated immune response in adult and aged C57BL/6J mice. *Am. J. Physiol.* 273, R1631–R1637.

Derting, T. L., and Compton, S. (2003). Immune response, not immune maintenance, is energetically costly in wild white-footed mice (*Peromyscus leucopus*). *Physiol. Biochem. Zool.* 76, 744–752.

DiAngelo, J. R., Bland, M. L., Bambina, S., Cherry, S., and Birnbaum, M. J. (2009). The immune response attenuates growth and nutrient storage in Drosophila by reducing insulin signaling. *Proc. Natl. Acad. Sci. U.S.A.* 106, 20853–20858.

Doeschl-Wilson, A., Sawalha, R., Gubbins, S., and Villanueva, B. (2009). Implications of conflicting associations of the prion protein (PrP) gene with scrapie susceptibility and fitness on the persistence of scrapie. *PLoS ONE* 4:e7970. doi: 10.1371/journal.pone.0007970

Donabedian, H. (2006). Nutritional therapy and infectious diseases: a two-edged sword. *Nutr. J.* 5, 21–31.

Duffy, M. A., and Forde, S. E. (2009). Ecological feedbacks and the evolution of resistance. *J. Anim. Ecol.* 78, 1106–1112.

Dunnington, E. A. (1990). "Selection and homeostasis," in *Proceedings of the 4th WCGALP* (Edinburgh, UK), 5–12.

Eady, S. J., Woolaston, R. R., Mortimer, S. I., Lewer, R. P., Raadsma, H. W., Swan, A. A., et al. (1996). Resistance to nematode parasites in Merino sheep: sources of genetic variation. *Aust. J. Agr. Res.* 47, 895–915.

Elia, M. (1992). "Organ and tissue contribution to metabolic rate," in *Energy Metabolism: Tissue Determinants and Cellular Corollaries,* eds J. M. McKinney and H. N. Tucker (New York, NY: Raven), 61–77.

Emanuelsson, U. (1988). Recording of production disease in cattle and possibilities for genetic improvements: a review. *Livest. Prod. Sci.* 20, 89–106.

Festa-Bianchet, M. (1989). Individual differences, parasites, and the costs of reproduction for bighorn ewes (*Ovis Canadensis*). *J. Anim. Ecol.* 58, 785–795.

Forbes, J. M. (2007). "Diet selection: practicalities," in *Voluntary Food Intake and Diet Selection in Farm Animals*, ed J. M. Forbes (Wallingford, UK: CABI Publishing), 453.

Fornoni, J., Nunez-Farfán, J., Valverde, P. L., and Rausher, M. D. (2004). Evolution of mixed strategies of plant defense allocation against natural enemies. *Evolution* 58, 1685–1695.

Freitak, D., Ots, I., Vanatoa, A., and Hõrak, P. (2003). Immune response is energetically costly in white cabbage butterfly pupae. *Proc. Biol. Sci.* 270(Suppl.), S220–S222.

French, S. S., DeNardo, D. F., and Moore, M. C. (2007). Trade-offs between the reproductive and immune systems: facultative responses to resources or obligate responses to reproduction? *Am. Nat.* 170, 79–89.

Friggens, N. C., and Van der Waaij, E. H. (2009). "Modelling of resource allocation patterns," in *Resource Allocation Theory Applied to Farm Animal Production*, ed W. M. Rauw (Wallingford, UK: CABI Publishing), 302–320.

Glazier, D. S. (2009a). "Resource allocation patterns," in *Resource Allocation Theory Applied to Farm Animal Production*, ed W. M. Rauw (Wallingford, UK: CABI Publishing), 22–43.

Glazier, D. S. (2009b). "Trade-offs," in *Resource Allocation Theory Applied to Farm Animal Production*, ed W. M. Rauw (Wallingford, UK: CABI Publishing), 44–60.

Gross, W. B., Siegel, P. B., and Pierson, F. W. (2002). Effects of genetic selection for high or low antibody response on resistance to a variety of disease challenges and the relationship of resource allocation. *Avian Dis.* 46, 1007–1010.

Haldane, J. B. S. (1949). Disease and evolution. *Ric. Sci. Suppl. A* 19, 68–76.

Han, P. F. S., and Smyth, J. R. Jr. (1972). The influence of growth rate on the development of Marek's disease in chickens. *Poult. Sci.* 51, 975–985.

Hanssen, S. A., Hasselquist, D., Folstad, I., and Erikstad, K. E. (2004). Costs of immunity: immune responsiveness reduces survival in a vertebrate. *Proc. Biol. Sci.* 271, 925–930.

Hasday, J. D., Fairchild, K. D., and Shanhotz, C. (2000). The role of fever in the infected host. *Microbes Infect.* 2, 1891–1904.

Hawkins, A. J. S. (1991). Protein turnover: a functional appraisal. *Funct. Ecol.* 5, 222–233.

Heringstad, B., Klemetsdal, G., and Ruane, J. (2000). Selection for mastitis resistance in dairy cattle: a review with focus on the situation in the Nordic countries. *Livest. Prod. Sci.* 64, 95–106.

Heringstad, B., Klemetsdal, G., and Steine, T. (2003). Selection responses for clinical mastitis and protein yield in two Norwegian dairy cattle selection experiments. *J. Dairy Sci.* 86, 2990–2999.

Hoeve, M. A., Mylonas, K. J., Fairlie-Clarke, K. J., Mahajan, S. M., Allen, J. E., and Graham, A. L. (2009). *Plasmodium chabaudi* limits early *Nippostrongylus brasiliensis*-induced pulmonary immune activation and Th2 polarization in co-infected mice. *BMC Immunol.* 10:60. doi: 10.1186/1471-2172-10-60

Houdijk, J. G. M., and Athanasiadou, S. (2003). "Direct and indirect effects of host nutrition on ruminant gastrointestinal nematodes," in *VI International Symposium on the Nutrition of Herbivores,* (Mérida, Mexico), 213–236.

Houdijk, J. G. M., Jessop, N. S., and Kyriazakis, I. (2001). Nutrient partitioning between reproductive and immune functions in animals. *Proc. Nutr. Soc.* 60, 515–525.

Howse, S. W., Blair, H. T., Garrick, D. J., and Pomroy, W. E. (1992). A comparison of internal parasitism in fleece-weight selected and control Romney sheep. *Proc. N.Z. Soc. Anim. Prod.* 52, 57–60.

Janeway, C. A., and Medzhitov, R. (2002). Innate immune recognition. *Annu. Rev. Immunol.* 20, 1–28.

Johnson, P. T. J., Rohr, J. R., Hoverman, J. T., Kellermanns, E., Bowerman, J., and Lunde, K. B. (2012). Living fast and dying of infection: host life history drives interspecific variation in infection and disease risk. *Ecol. Lett.* 15, 235–242.

Jokela, J., Schmid-Hempel, P., and Rigby, M. C. (2000). Dr. Pangloss restrained by the Red Queen – steps towards a unified defence theory. *OIKOS* 89, 267–274.

Katona, P., and Katona-Apte, J. (2008). The interaction between nutrition and infection. *Clin. Infect. Dis.* 46, 1582–1588.

Kelley, K. W. (1980). Stress and immune function: a bibliographic review. *Ann. Rech. Vét.* 11, 445–478.

Klasing, K. C., Laurin, D. E., Peng, R. K., and Fry, D. M. (1987). Immunologically mediated growth depression in chicks: influence of feed intake, corticosterone and interleukin-1. *J. Nutr.* 117, 1629–1637.

Kluger, M. J., Kozak, W., Conn, C. A., Leon, L. R., and Soszynski, D. (1998). Role of fever in disease. *Ann. N.Y. Acad. Sci.* 856, 224–233.

Knap, P. W. (2005). Breeding robust pigs. *Aust. J. Exp. Agr.* 45, 763–773.

Knap, P. W. (2009). "Robustness," in *Resource Allocation Theory Applied to Farm Animal Production*, ed W. M. Rauw (Wallingford, UK: CABI Publishing), 288–301.

Knap, P. W., and Bishop, S. C. (1996). "Relationships between genetic change and infectious disease in domestic livestock," in *The Challenge of Genetic Change in Animal Production*, eds W. G. Hill, S. C. Bishop, B. McGuirk, J. C. McKay, G. Simm, and A. J. Webb (Edinburgh, UK: British Society of Animal Science). Available online at: http://www.bsas.org.uk/publs/genchng/paper8.pdf

Knox, M., and Steel, J. (1996). Nutritional enhancement of parasite control in small ruminant production systems in developing countries of south-east Asia and the Pacific. *Int. J. Parasitol.* 26, 963–970.

Koenen, M. E., Boonstra-Blom, A. G., and Jeurissen, S. H. M. (2002). Immunological differences between layer- and broiler-type chickens. *Vet. Immunol. Immunopathol.* 89, 47–56.

Kogut, M. H. (2009). Impact of nutrition on the innate immune response to infection in poultry. *J. Appl. Poultry Res.* 18, 111–124.

Kraaijeveld, A. R., and Godfray, H. C. J. (2008). Selection for resistance to a fungal pathogen in *Drosphila melanogaster*. *Heredity* 100, 400–406.

Kyriazakis, I., and Houdijk, J. (2006). Immunonutrition: nutritional control of parasites. *Small Ruminant Res.* 62, 79–82.

Kyriazakis, I., Oldham, J. D., Coop, R. L., and Jackson, F. (1994). The effect of subclinical intestinal nematode infection on the diet selection of growing sheep. *Br. J. Nutr.* 72, 665–677.

Kyriazakis, I., Tolkamp, B. J., and Hutchings, M. R. (1998). Towards a functional explanation for the occurrence of anorexia during parasitic infections. *Anim. Behav.* 56, 265–274.

Lamont, S. J., Pinard-Van der Laan, M. H., Cahaner, A., Van der Poel, J. J., and Parmentier, H. K. (2003). "Selection for disease resistance: direct selection on the immune response," in *Poultry Genetics, Breeding and Biotechnology*, eds W. M. Muir and S. E. Aggrey (Wallingford, UK: CABI Publishing), 399–418.

Lee, K. A. (2006). Linking immune defenses and life history at the levels of the individual and the species. *Integr. Comp. Biol.* 46, 1000–1015.

Lee, K. A., Wikelski, M., Robinson, W. D., Robinson, T. R., and Klasing, K. C. (2008). Constitutive immune defences correlate with life-history variables in tropical birds. *J. Anim. Ecol.* 77, 356–363.

Lee, K. P., Cory, J. S., Wilson, K., Raubenheimer, D., and Simpson, S. J. (2006). Flexible diet choice offsets protein costs of pathogen resistance in a caterpillar. *Proc. Biol. Sci.* 273, 823–829.

Le Floc'h, N., Melchior, D., and Obled, C. (2004). Modifications of protein and amino acid metabolism during inflammation and immune sytem

activation. *Livest. Prod. Sci.* 87, 37–45.

Leroi, A. M. (2001). Molecular signals versus the loi de balancement. *Trends Ecol. Evol.* 16, 24–29.

Lobley, G. E. (2003). Protein turnover – what does it mean for animal production? *Can. J. Anim. Sci.* 83, 327–340.

Lochmiller, R. L., and Deerenberg, C. (2000). Trade-offs in evolutionary immunology: just what is the cost of immunity? *OIKOS* 88, 87–98.

Martin, A., Dunnington, E. A., Gross, W. B., Briles, W. E., Briles, R. W., and Siegel, P. B. (1990). Production traits and alloantigen systems in lines of chickens selected for high or low antibody responses to sheep erythrocytes. *Poult. Sci.* 69, 871–878.

Martin, L. B., Scheuerlein, A. L., and Wikelski, M. (2003). Immune activity elevates energy expenditure of house sparrows: a link between direct and indirect costs? *Proc. Biol. Sci.* 270, 153–158.

Mauck, R. A., Matson, K. D., Philipsborn, J., and Ricklefs, R. E. (2005). Increase in the constitutive innate humoral immune system in Leach's Storm-Petrel (*Oceanodroma leucorhoa*) chicks is negatively correlated with growth rate. *Funct. Ecol.* 19, 1001–1007.

Mauricio, R., Rausher, M. D., and Burdick, D. S. (1997). Variation in the defense strategies of plants: are resistance and tolerance mutually exclusive? *Ecology* 78, 1301–1311.

McDade, T. W. (2005). Life history, maintenance, and the early origins of immune function. *Am. J. Hum. Biol.* 17, 81–94.

McFall-Ngai, M. (2007). Care for the community. *Nature* 445, 11.

McNamara, J. M., and Buchanan, K. L. (2005). Stress, resource allocation, and mortality. *Behav. Ecol.* 16, 1008–1017.

Miller, L. L., Siegel, P. B., and Dunnington, E. A. (1992). Inheritance of antibody response to sheep erythrocytes in lines of chickens divergently selected for 56-day body weight and their crosses. *Poult. Sci.* 71, 47–52.

Moberg, G. P. (2000). "Biological response to stress: implications for animal welfare," in *The Biology of Animal Stress*, eds G. P. Moberg and J. A. Mench (Wallingford, UK: CABI Publishing), 1–22.

Møller, A. P., Christe, Ph., Erritzøe, J., and Mavarez, J. (1998). Condition, disease and immune defence. *OIKOS* 83, 301–306.

Møller, A. P., and Saino, N. (2004). Immune response and survival. *OIKOS* 104, 299–304.

Mormède, P., Foury, A., Terenina, E., and Knap, P. W. (2010). Breeding for robustness: the role of cortisol. *Animal* 5, 651–657.

Moreno, J., Sanz, J. J., and Arriero, E. (1999). Reproductive effort and T-lymphocyte cell-mediated immunocompetence in female pied flycatchers *Ficedula hypoleuca*. *Proc. Biol. Sci.* 266, 1105–1109.

Morris, C. A., Vlassoff, A., Bisset, S. A., Baker, R. L., Watson, T. G., West, C. J., et al. (2000). Continued selection of Romney sheep for resistance or susceptibility to nematode infection: estimates of direct and correlated responses. *Anim. Sci.* 70, 17–27.

Nestor, K. E., Noble, D. O., Zhu, J., and Moritsu, Y. (1996a). Direct and correlated responses to long-term selection for increased body weight and egg production in turkeys. *Poult. Sci.* 75, 1180–1191.

Nestor, K. E., Saif, Y. M., Zhu, J., and Noble, D. O. (1996b). Influence of growth selection in turkeys on resistance to Pasteurella multocida. *Poult. Sci.* 75, 1161–1163.

Nilsson, J. Å. (2003). Ectoparasitism in marsh tits: costs and functional explanations. *Behav. Ecol.* 14, 175–181.

Nordling, D., Andersson, M., Zohari, S., and Gustafsson, L. (1998). Reproductive effort reduces specific immune response and parasite resistance. *Proc. Biol. Sci.* 265, 1291–1298.

Oddy, V. H., Speck, P. A., Warren, H. M., and Wynn, P. C. (1995). Protein metabolism in lambs from lines divergently selected for weaning weight. *J. Agric. Sci.* 124, 129–137.

Ots, I., Kerimov, A. B., Ivankina, E. V., Ilyina, T. A., and Hõrak, P. (2001). Immune challenge affects basal metabolic activity in wintering great tits. *Proc. Biol. Sci.* 268, 1175–1181.

Panda, A. K., Raju, M. V. L. N., Rama Rao, S. V., Shyam Sunder, G., and Reddy, M. R. (2010). Effect of post-hatch feed deprivation on growth, immune organ development and immune competence in broiler chickens. *Anim. Nutr. Feed Techn.* 10, 9–17.

Pirlet, K., and Arthur-Goettig, A. (1999). Maintaining life and health by natural selection of protein molecules. *J. Theor. Biol.* 201, 75–85.

Povey, S., Cotter, S. C., Simpson, S. J., Lee, K. P., and Wilson, K. (2009). Can the protein costs of bacterial resistance be offset by altered feeding behavior? *J. Anim. Ecol.* 78, 437–446.

Princiotta, M. F., Finzi, D., Qian, S. B., Gibbs, J., Schuchmann, S., Buttgereit, F., et al. (2003). Quantitating protein synthesis, degradation, and endogenous antigen processing. *Immunity* 18, 343–354.

Pryce, J. E., Veerkamp, R. F., Thomposon, R., Hill, W. G., and Simm, G. (1997). Genetic aspects of common health disorders and measures of fertility in Holstein Friesian dairy cattle. *Anim. Sci.* 3, 353–360.

Qureshi, M. A., and Havenstein, G. B. (1994). A comparison of the immune performance of a 1991 commercial broiler with a 1957 randombred strain when fed 'typical' 1957 and 1991 broiler diets. *Poult. Sci.* 73, 1805–1812.

Råberg, L., Sim, D., and Read, A. F. (2007). Disentangling genetic variation for resistance and tolerance to infectious diseases in animals. *Science* 318, 812–814.

Råberg, L., Vestberg, M., Hasselquist, D., Holmdahl, R., Svensson, E., and Nilsson, J. Å. (2002). Basal metabolic rate and the evolution of the adaptive immune system. *Proc. Biol. Sci.* 269, 817–821.

Rauw, W. M. (2007). Physiological consequences of selection for increased performance. *Proc. Assoc. Advmt. Anim. Breed. Genet.* 17, 240–247.

Rauw, W. M. (2009). "Introduction," in *Resource Allocation Theory Applied to Farm Animal Production*, ed W. M. Rauw (Wallingford, UK: CABI Publishing), 1–21.

Rauw, W. M. (2012). "Feed efficiency and animal robustness," in *Feed Efficiency in the Beef Industry*, ed R. A. Hill (West Sussex, UK: Wiley-Blackwell), 105–122.

Rauw, W. M., Kanis, E., Noordhuizen-Stassen, E. N., and Grommers, F. J. (1998). Undesirable side effects of selection for high production efficiency in farm animals: a review. *Livest. Prod. Sci.* 56, 15–33.

Raynal-Ljutovac, K., Pirisi, A., de Crémoux, R., and Gonzalo, C. (2007). Somatic cells of goat and sheep milk: analytical, sanitary, productive and technological aspects. *Small Ruminant Res.* 68, 126–144.

Reeds, P. J., and Jahoor, F. (2001). The amino acid requirement of disease. *Clin. Nutr.* 20, 15–22.

Restif, O., and Koella, J. C. (2004). Concurrent evolution of resistance and tolerance to pathogens. *Am. Nat.* 164, E90–E102.

Richardson, E. C., and Herd, R. M. (2004). Biological basis for variation in residual feed intake in beef cattle. 2. Synthesis of results following divergent selection. *Aust. J. Exp. Agric.* 44, 431–440.

Ricklefs, R. E., and Wikelski, M. (2002). The physiology/life-history nexus. *Trends Ecol. Evol.* 17, 462–468.

Rigby, M. C., Hechinger, R. F., and Stevens, L. (2002). Why should parasite resistance be costly? *Trends Parasitol.* 18, 116–120.

Roff, D. A. (2007). Contributions of genomics to life-history theory. *Nat. Rev. Genet.* 8, 116–125.

Roy, B. A., and Kirchner, J. W. (2000). Evolutionary dynamics of pathogen resistance and tolerance. *Evolution* 54, 51–63.

Rupp, R., and Boichard, D. (2003). Genetics of resistance to mastitis in dairy cattle. *Vet. Res.* 34, 671–688.

Rupp, R., and Foucras, G. (2010). "Genetics of mastitis in dairy ruminants," in *Breeding for Disease Resistance in Farm Animals*, eds S. C. Bishop, R. F. E. Axford, F. W. Nicholas, and J. B. Owen (Wallingford, UK: CABI Publishing), 183–212.

Sacco, R. E., Saif, Y. M., Nestor, K. E., Anthony, N. B., Emmerson, D. A., and Dearth, R. N. (1991). Genetic variation in resistance of turkeys to experimental challenge with pasteurella multocida. *Avian Dis.* 35, 950–954.

Saif, Y. M., Nestor, K. E., Dearth, R. N., and Renner, P. A. (1984). Possible genetic variation in resistance of turkeys to erysipelas and fowl cholera. *Avian Dis.* 28, 770–773.

Sakaguchi, S., Yamaguchi, T., Nomura, T., and Ono, M. (2008). Regulatory T cells and immune tolerance. *Cell* 133, 775–787.

Sandland, G. J., and Minchella, D. J. (2003). Costs of immune defense: an enigma wrapped in an environmental cloak? *Trends Parasitol.* 19, 571–574.

Saper, C. B., and Breder, C. D. (1994). The neurological basis of fever. *N. Engl. J. Med.* 330, 1880–1886.

Sawalha, R. M., Brotherstone, S., Conington, J., and Villanueva, B. (2007). Lambs with scrapie susceptible genotypes have higher postnatal survival. *PLoS ONE* 11:e1236. doi: 10.1371/journal.pone.0001236

Schluter, D., Price, T. D., and Rowe, L. (1991). Conflicting selection pressures and life-history trade-offs. *Proc. Biol. Sci.* 246, 11–17.

Schmid-Hempel, P. (2003). Variation in immune defence as a question of evolutionary ecology. *Proc. Biol. Sci.* 270, 357–366.

Schneider, D. S., and Ayres, J. S. (2008). Two ways to survive infection: what

resistance and tolerance can teach us about treating infectious diseases. *Nat. Rev.* 8, 889–895.

Segerstrom, S. C. (2007). Stress, energy, and immunity: an ecological view. *Curr. Dir. Phychol. Sci.* 16, 326–330.

Selye, H. (1953). The present state of stress conception. *Munch. Med. Wochenschr.* 95, 426–433.

Shubber, A. H., Lloyd, S., and Soulsby, E. J. L. (1981). Infection with gastrointestinal helminths. Effect of lactatin and meternal transfer of immunity. *Z. Parasitenkd.* 65, 181–189.

Simms, E. L. (2000). Defining tolerance as a norm of reaction. *Evol. Ecol.* 14, 563–570.

Speakman, J. R. (2008). The physiological costs of reproduction in small mammals. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 363, 375–398.

Spurlock, M. E. (1997). Regulation of metabolism and growth during immune challenge: an overview of cytokine function. *J. Anim. Sci.* 75, 1773–1783.

Stear, M. J., Bishop, S. C., Mallard, B. A., and Raadsma, H. (2001). The sustainability, feasibility and desirability of breeding livestock for disease resistance. *Res. Vet. Sci.* 71, 1–7.

Svensson, E., and Råberg, L. (2010). Resistance and tolerance in animal enemy-victim coevolution. *Trends Ecol. Evol.* 25, 267–274.

Svensson, E., Råberg, L., Koch, C., and Hasselquist, D. (1998). Energetic stress, immunosuppression and the cost of an antibody response. *Funct. Ecol.* 12, 912–919.

Sykes, A. R. (2010). Host immune responses to nematodes: benefit or cost? Implications for future development of sustainable methods of control. *Rev. Bras. Zoot.* 39, 376–382.

Sykes, A. R., and Coop, R. L. (2001). Interactions between nutrition and gastrointestinal parasitism in sheep. *N.Z. Vet. J.* 49, 222–226.

Tavernarakis, N., and Driscoll, N. (2002). Caloric restriction and lifespan: a role for protein turnover? *Mech. Ageing Dev.* 123, 215–229.

Thomas, F. M., Pym, R. A., and Johnson, R. J. (1991). Muscle protein turnover in chickens selected for increased growth rate, food consumption or efficiency of food utilization: effects of genotype and relationship to plasma IGF-I and growth hormone. *Br. Poult. Sci.* 32, 363–376.

Tomkins, A. M. (1986). Protein-energy malnutrition and risk of infection. *Proc. Nutr. Soc.* 45, 289–304.

Tort, L. (2011). Stress and immune modulation in fish. *Dev. Comp. Immunol.* 35, 1366–1375.

Tsai, H. J., Saif, Y. M., Nestor, K. E., Emmerson, D. A., and Patterson, R. A. (1992). Genetic variation in resistance of turkeys to experimental infection with Newcastle disease virus. *Avian Dis.* 36, 561–565.

Van der Most, P. J., De Jong, B., Permetier, H. K., and Verhulst, S. (2011). Trade-off between growth and immune function: a meta-analysis of selection experiments. *Funct. Ecol.* 25, 74–80.

Van Heugten, E. V., and Spears, J. W. (1997). Immune response and growth of stressed weanling pigs fed diets supplemented with organic or inorganic forms of chromium. *J. Anim. Sci.* 75, 409–416.

Van Houtert, M. F. J., Barger, I. A., Steel, J. W., Windon, R. G., and Emergy, D. L. (1995). Effects of dietary protein intake on responses of young sheep to infection with *Trichostrongylus colubriformis*. *Vet. Parasitol.* 56, 163–180.

Van Straalen, N. M., and Roelofs, D. (2006). *An Introduction to Ecological Genomics*. Oxford, UK: Oxford University Press.

Veerkamp, R. F., Windig, J. J., Calus, M. P. L., Ouweltjes, W., De Haas, Y., and Beerda, B. (2009). "Selection for high production in dairy cattle," in *Resource Allocation Theory Applied to Farm Animal Production*, ed W. M. Rauw (Wallingford, UK: CABI Publishing), 243–260.

Verhulst, S., Riedstra, B., and Wiersma, P. (2005). Brood size and immunity costs in zebra finches *Taeniopygia guttata*. *J. Avian Biol.* 36, 22–30.

Via, S., Gomulkiewicz, R., De Jong, G., Scheiner, S. M., Schlichting, C. D., and Van Tienderen, P. H. (1995). Adaptive phenotypic plasticity: consensus and controversy. *Trends. Ecol. Evol.* 10, 212–217.

Viney, M. E., Riley, E. M., and Buchanan, K. L. (2005). Optimal responses: immunocompetence revisited. *Trends Ecol. Evol.* 20, 665–669.

Walsh, T. S. (2007). "The metabolic response to injury," in *Principles and Practice of Surgery,* eds O. J. Garden, A. W. Bradbury, and J. Forsythe (Scotland: Churchill Livingstone), 2–9.

Warner, C. M., Meeker, D. L., and Rothschild, M. F. (1987). Genetic control of immune responsiveness: a review of its use as a tool for selection for disease resistance. *J. Anim. Sci.* 64, 394–406.

Webster Marketon, J. I., and Glaser, R. (2008). Stress hormones and immune function. *Cell. Immunol.* 252, 16–26.

Weiner, J. (1992). Physiological limits to sustainable energy budgets in birds and mammals: ecological implications. *Trends Ecol. Evol.* 7, 384–388.

Wilkie, B., and Mallard, B. (1999). Selection for high immune response: an alternative approach to animal health maintenance? *Vet. Immunol. Immunopathol.* 72, 231–235.

Williams, N. H., Stahly, T. S., and Zimmerman, D. R. (1997). Effect of chronic immune system activation on the rate, efficiency, and composition of growth and lysine needs of pigs fed from 6 to 27 kg. *J. Anim. Sci.* 75, 2463–2471.

Xu, Y. C., Yang, D. B., and Wang, D. H. (2012). No evidence for a trade-off between reproductive investment and immunity in a rodent. *PLoS ONE* 7:e37182. doi: 10.1371/journal.pone.0037182

Yewdell, J. W. (2001). Not such a dismal science: the economics of protein synthesis, folding, degradation and antigen processing. *Trends Cell Biol.* 11, 294–297.

Zhao, X., and Lacasse, P. (2008). Mammary tissue damage during bovine mastitis: causes and control. *J. Anim. Sci.* 86, 57–65.

Zuk, M., and Stoehr, A. M. (2002). Immune defense and host life history. *Am. Nat.* 160, S9–S22.

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A consideration of resistance and tolerance for ruminant nematode infections

Stephen C. Bishop*

Genetics and Genomics, Royal (Dick) School of Veterinary Studies, The Roslin Institute, University of Edinburgh, Edinburgh, UK

Edited by:
Andrea B. Doeschl-Wilson, The University of Edinburgh, UK

Reviewed by:
Dirk-Jan De Koning, Swedish University of Agricultural Sciences, Sweden
Shaolin Wang, University of Virginia, USA

***Correspondence:**
Stephen C. Bishop, Genetics and Genomics, Royal (Dick) School of Veterinary Studies, The Roslin Institute, University of Edinburgh, Midlothian, EH25 9RG, UK.
e-mail: stephen.bishop@roslin.ed.ac.uk

Debates on the relative merits of resistance (the ability of the host to control the parasite lifecycle) and tolerance (the net impact of infection on host performance) are often lively and unhindered by data or evidence. Resistance generally shows continuous, heritable variation but data are sparser for tolerance, the utility of which will depend upon the disease prevalence. Prevalence is a function of group mean resistance and infection pressure, which itself is influenced by mean resistance. Tolerance will have most value for endemic diseases with a high prevalence but will be of little value for low prevalence diseases. The conditionality of tolerance on infection status, and hence resistance, makes it difficult to estimate independently of resistance. Tolerance is potentially tractable for nematode infections, as the prevalence of infection is ca. 100% in animals grazing infected pasture, and infection level can be quantified by faecal egg count (FEC). Whilst individual animal phenotypes for tolerance are difficult to estimate, breeding values are estimable if related animals graze pastures of different contamination levels. Selection for resistance, i.e., FEC, provides both direct and indirect benefits from ever decreased pasture contamination and hence decreased infectious challenge. Modeling and experimental studies have shown that such reductions in pasture contamination may lead to substantially increased performance. It is proposed that selection goals addressing nematode infections should include both resistance and performance under challenging conditions. However, there may be benefits from exploiting large datasets in which sires are used across cohorts differing in infection level, to further explore tolerance. This may help to customise breeding objectives, with tolerance given greater weight in heavily parasitized environments.

Keywords: resilience, sheep, worms, animal genetics, epidemiology

INTRODUCTION

This paper aims to consider the relative definitions of resistance and tolerance, as applied to host genetic resistance to disease in livestock, determine the situations when resistance and tolerance are useful breeding goals, and apply the concepts discussed to nematode infections in ruminants. Currently there is considerable debate amongst livestock geneticists on the relative utility of resistance and tolerance when considering the term "disease resistance"; such debates are often unhindered by data or evidence and are even unhindered by a consistent logical thread in the argument. Curiously a parallel debate on the merits of tolerance and resistance has been conducted within the ecological and immunological communities (e.g., Råberg et al., 2007, 2009). However, there has been little cross fertilization between these different groups of researchers. The debate on the relative merits of resistance and tolerance is particularly apposite now, as disease resistance is becoming an ever more ubiquitous goal in many breeding programs and is invariably nominated by breeders as a high priority trait. Further, with the ready availability of DNA from populations of animals that have faced epidemic challenges, genomic selection (albeit with low precision) is now becoming an option for diseases that hitherto would have been difficult to incorporate into breeding programs.

From consideration of literature on disease resistance (from a livestock viewpoint) it is apparent that different authors have different interpretations of the term "resistance". For example, common usage is to define resistance in terms of susceptibility to infection *per se*, i.e., liability to becoming infected when faced with an infectious challenge of a parasite or pathogen, with animals that are less susceptible being more resistant. However, this definition does not hold for nematode infections, where faecal egg count (FEC) is often used as the indicator of relative resistance and FEC may be thought of summarizing the net outcome of the host–parasite interaction. The issue of trait definition for resistance has even been avoided on occasions. For example Boddicker et al. (2012), in a study aiming to find QTL for resistance to porcine reproductive and respiratory syndrome (PRRS), simply described their trait (viraemia following infection) as a measure of host response to infection.

The trait definition problem can be clarified to some extent by generalizing the definitions to encapsulate the trait biology, as outlined by Bishop and Stear (2003). Defining infection as the colonization of a host animal by a parasite (or pathogen) and disease as the side effects of infection, these authors then defined resistance as the ability of the individual host to control or influence the parasite (pathogen) lifecycle, and tolerance as the net

impact of infection on the performance of host animal, i.e., the disease side-effects. These definitions are consistent with those used elsewhere in this Special Topic. Definitions as broad as this allow the concepts of resistance and tolerance to be applied to any disease, and to be applied equally to any aspect of the host–parasite (pathogen) interaction or any outcome of infection. Full definitions of the terms used in this paper to describe impacts of infection on individual hosts and in populations are shown in **Box 1**, along with a diagrammatic representation of these terms.

This review article considers the wider implications of resistance and tolerance, when applied to any infectious disease, with a particular focus on nematode infections in ruminants. It is assumed that for most diseases host–parasite interactions are complex and under partial genetic control (e.g., Davies et al., 2009). Further, it is assumed that the complexity of the host–parasite interactions leads to variation in resistance being polygenic in most (but not all) cases.

DEFINING TOLERANCE IN AN EPIDEMIOLOGICAL CONTEXT

The relative merits of resistance and tolerance as breeding goals depend upon the nature and epidemiology of the disease. Self-evidently, tolerance is only expressed when animals are infected, therefore the value of tolerance depends, amongst other factors, on the epidemiology of the disease. Two factors come into play, the interpretation (and hence utility) of tolerance and the estimation of tolerance.

INTERPRETATION AND UTILITY OF TOLERANCE

Tolerance can only be expressed once an animal has become infected. Thus, with a given prevalence (p) of infection (see **Box 1**), a proportion p of the population will express tolerance and 1-p will not. As p approaches unity, tolerance becomes a useful concept, however as p falls to levels such that a large proportion of animals are not infected, its utility becomes questionable.

Box 1 | Definitions used in paper.

Resistance

The ability of the host animal to exert control over the parasite or pathogen lifecycle. *Measurements which indicate level of parasite burden are often considered to be indicators of resistance. Such traits include faecal egg count, viraemia, or bacterial load in animals infected with nematodes, viruses, or bacteria, respectively.*

Tolerance

The net impact on performance of a given level of infection. *Measurement of tolerance is logistically difficult as discussed here and by Doeschl-Wilson et al. (2012a,b).*

Resilience

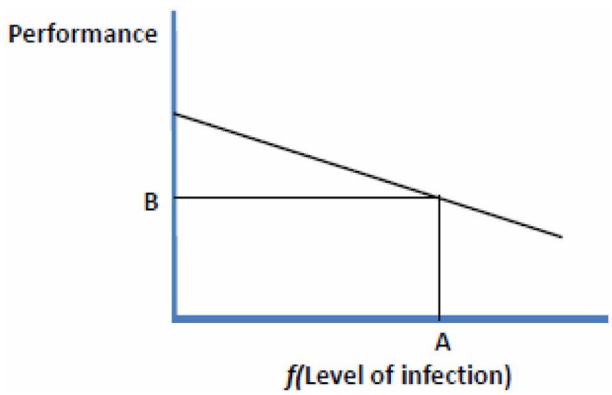
The productivity of an animal in the face of infection. *Resilience is often measured simply as performance in an infected environment, however indirect measurements such as treatment requirements are sometimes used as a proxy.*

Prevalence

The proportion of the host population that is infected or diseased at a specific point in time.

Incidence

The number of new cases that arise in a population over a specified time period. *Incidence is a rate parameter and is often incorrectly confused with prevalence.*



The figure shows a schematic representation of performance and level of infection, or some function that linearises the relationship between level of infection and performance. The regression slope represents **Tolerance**, point A indicates **Resistance** and point B represents **Resilience**.

Let us assume initially that tolerance is estimable at the individual animal level, and hence that infection status of individual animals is known. The information available for tolerance within a population will depend upon many factors, including those which determine the true prevalence of infection, and those which determine the observed prevalence, conditional on the true prevalence. The latter is largely a function of the accuracy of the diagnostic tests, i.e., the specificity (S_p, this being the probability that a truly *uninfected* individual is classified by the diagnostic test as *uninfected)* and sensitivity (S_e, this being the probability that a truly *infected* individual is classified by the diagnostic test as *infected)*. As shown by Bishop and Woolliams (2010), the regression of observed on true prevalence is $p' = (1 - S_p) + (S_p + S_e - 1)p$, hence imperfect diagnostic tests will reduce heritabilities by a factor $(S_p + S_e - 1)^2$, and estimated SNP effects by a factor $(S_p + S_e - 1)$.

Factors affecting true prevalence are more complex, depending upon the force of infection (Anderson and May, 1991). Thus, prevalence is influenced both directly and indirectly (through the infectivity of infected animals) by the mean resistance of the population. Therefore, the utility of tolerance is partly dependent on the mean resistance of the population, with tolerance becoming more valuable as mean resistance is decreased. However, at the individual animal level, and assuming that resistance to infection is heritable, it is the least resistant (or most susceptible) animals that are the most likely to become infected, and hence the most likely to yield information on tolerance. Thus, the expression of tolerance is conditional upon the individual animal's resistance to infection.

When applying these concepts to deriving breeding goals, the first point to note is that tolerance is most useful when p approaches unity. As soon as p drops substantially below unity, then resistance must also be included in the breeding goal, else there is a risk that selection pressure is targeted toward the least resistant animals in the population and away from those that are more valuable from a disease control perspective, *viz.* the more resistant animals.

A further point is that tolerance should not be considered as a breeding goal in situations where control of transmission of infection is paramount. Most obviously this applies to zoonotic infections, i.e., infections harbored by animals that cause disease in humans, but it applies also to situations where other populations surrounding our target flock/herd are notably susceptible to infection.

ESTIMATION OF TOLERANCE

Following the definitions used by Simms (2000) and adapted for the impact of infectious diseases on animal performance by Kause (2011), we may define tolerance for the *i*th animal as the regression slope (b_i) in the relationship: $Y_i = Y_{0i} + b_i f(Ii)$, where Y_i is the observed performance, Y_{0i} is performance when the animal is uninfected, Ii is the level of infection (pathogen/parasite burden) of the animal and $f(x)$ is some function which makes the relationship between pathogen burden and decline in performance approximately linear. This is shown diagrammatically in **Box 1**.

Two features of this relationship are important. Firstly, at the population level the (genetic) covariance of tolerance and performance under non-infected conditions is important [i.e., $\mathrm{Cov}(Y_0, b)$]; as one would not want decreased performance under non-infectious conditions to be an unintended consequence of selection for improved tolerance. Secondly, tolerance is likely to be difficult to measure at the individual animal level in many circumstances, as measurements of performance at two or more different levels of infection will be required, on the same animal. This concept has been explored in detail by Doeschl-Wilson et al. (2012b, this volume), and these authors propose some novel analytical solutions. However, it is difficult to envisage how individual animal tolerance could be estimated in traits expressed over a short duration or only once (such as survival, longevity, growth rate over defined time periods, or carcass characteristics). On the other hand, for traits expressed repeatedly by adult animals (such as reproductive performance, lactation traits, or fiber production) measurement may be feasible.

Simply measuring productivity of animals under disease challenging conditions is seen as a desirable breeding goal in many circumstances and by many breeders, however this does not equate to tolerance. This trait is a composite of productivity under uninfected conditions, resistance (which affects pathogen/parasite level) and tolerance, further complicated by possible covariances between these traits. Also, provided that there is genetic variation in resistance, then this trait will show genotype by environment interactions as the force of infection changes.

PARASITE/PATHOGEN COEVOLUTION RISKS

As reviewed by Råberg et al. (2009), it has been long argued that tolerance places less selective pressure on the pathogen to evolve than resistance, hence tolerance should be a more sustainable selection criterion. Further, in an evolutionary context, selective pressure on mutations enhancing tolerance (where the "performance" trait is fitness) will tend to fixation (Roy and Kirchner, 2000), whereas selective pressure on mutations for resistance will decrease as the allele frequency increases due to the genetic equivalent of herd immunity. These arguments are backed empirically by the observation that livestock living in areas with high infectious disease challenge generally tend to be tolerant of infection rather than resistant, a prime example being trypanotolerance.

These arguments are, however, rather simplistic and may require modification. Firstly, these arguments ignore a third factor in the arms race, *viz.* parasite virulence. When all three traits are put together, expected outcomes over co-evolutionary time periods are complex and depend on assumed relationships amongst the traits (Carval and Ferriere, 2010). However the problem can be simplified by acknowledging that full co-evolutionary models are not necessary, as in the livestock context genetic changes in host animals are controlled and, in most cases, likely to be relatively small. To my knowledge there are no robust theoretical considerations of pressures placed on pathogen evolution through selection for resistance or tolerance, however analogous studies have been done for parasite evolution risks arising from vaccines with different modes of action (Gandon et al., 2001). Considering anti-malarial vaccines, and under the assumptions of their model, vaccines affecting susceptibility to infection, infectivity, and tolerance had somewhat similar predicted effects on parasite virulence evolution, whereas those affecting parasite

proliferation led the parasite to evolve toward markedly greater virulence. In summary, it is likely that some aspects of resistance place greater selection pressure on the pathogen to evolve than tolerance, however this argument should be stated in shades of gray rather than black and white.

SYNOPSIS

Tolerance may be a useful concept for some diseases, depending upon the epidemiological context. Further, it provides an advantage over resistance insofar as tolerance of infection, in many circumstances, may place less selective pressure on the pathogen than resistance. However, there are a number of caveats to beware of when considering tolerance as a breeding goal. First, it is not desirable for zoonotic infections. Secondly, its value depends upon the prevalence of infection, decreasing as prevalence decreases. Thirdly, as prevalence decreases, there is a risk that selection intensity can only be achieved for the least resistant animals, implying that tolerance should never be decoupled from resistance within a breeding goal. Finally, the range of traits for which tolerance can be easily or unambiguously estimated at an individual animal level may be limited to those repeatedly expressed by adult animals over the course of their lifetime.

One class of diseases that meets most of the criteria necessary for tolerance to be a feasible selection goal is nematode infections, particularly in ruminants. This paper will now focus on the application of tolerance to nematode infections.

APPLICATION TO NEMATODE INFECTIONS

INTRODUCTION TO THE ISSUE

Gastrointestinal nematode parasite infections, particularly of ruminants, are probably the class of disease with the greatest impact upon animal health and productivity, particularly in developing countries where they have a large impact on the livelihoods of livestock keepers (Perry et al., 2002). Furthermore, they also represent an important disease issue in developed countries, especially in the sheep and goat sector. However, nematodes represent a threat to any extensively kept livestock species.

Much work has been done quantifying genetic variation for many aspects of the host response to infection in small ruminants [see summary by Bishop and Morris (2007)], and heritable variation is nearly always observed. Further, numerous studies have shown that selection for resistance is possible and effective in both sheep (Woolaston and Piper, 1996; Woolaston and Windon, 2001; Morris et al., 2005; Karlsson and Greeff, 2006; Kemper et al., 2010a) and goats (Vagenas et al., 2002), and indeed it is now widely implemented in several countries, notably New Zealand and Australia. Such selection should reduce costs of parasitism and increase the shelf-life of anthelmintics in the face of widespread evolution of anthelmintic resistance in nematodes (Waller, 1997; Jackson and Coop, 2000).

Despite the apparent success in breeding sheep for resistance to nematodes, considerable debate still exists on the best phenotype to use for selection, i.e., should it be resistance, tolerance or resilience? NB resilience may be thought of as the productivity of an animal in the face of infection (see **Box 1**). I have previously discussed this topic (Bishop, 2012), however here I consider it further. It should firstly be pointed out that nematode infections do lend themselves, in principle, to breeding for tolerance or some related trait as the prevalence of infection is invariably close to 100% and nematode infections are not zoonotic.

DEFINITION AND CONSEQUENCES OF SELECTABLE TRAITS

The indicator trait most conveniently used to describe resistance to nematode infections is FEC. This is a composite trait, being the product of (female) worm burden and worm fecundity. Because it is invariably heavily right-skewed, it is often log-transformed prior to analysis, hence we might expect the heritability of FEC to be close to the average of the heritabilities for worm burden and worm fecundity. These are difficult traits to measure, however when all three traits have been measured this expectation does hold true (Stear et al., 1997; Davies et al., 2005). Whilst FEC may be a good indicator of worm burden for nematode species such as *Haemonchus contortus* and *Trichostrongylus colubriformis*, for *Teladorsagia circumcincta* this relationship breaks down at high worm burdens due to density-dependent constraints on worm fecundity (Bishop and Stear, 2000).

More generally, many measurements have been used to quantify variation in impacts of nematode infections on host animals, and these have previously (Bishop, 2012) been classified as follows: (1) measures of resistance: FEC, worm burden, worm size, and fecundity; (2) measures of immune responses: e.g., eosinophilia, antibodies such as IgA, IgG, IgM; (3) measures of impact of infection: e.g., anaemia (as measured by packed cell volume (PCV) or eyelid color), pepsinogen or fructosamine concentrations; (4) various direct and indirect measures of resilience: including growth rate, anthelmintic requirements ("the age at which a first post-weaning anthelmintic treatment is required to maintain acceptable growth in lambs grazing nematode-contaminated pasture," Morris et al., 2010) and anaemia following *H. contortus* infection (Baker et al., 2003). Clearly categories (3) and (4) overlap in their definitions.

Selecting for increased resistance, i.e., decreased FEC, has an additional advantage of leading to both direct and indirect (epidemiological) benefits resulting from ever decreased pasture contamination and hence decreased infectious challenge. Several modeling studies have shown that whilst the direct impacts of selection for reduced FEC on performance traits depend on the genetic correlations between traits (e.g., Vagenas et al., 2007; Doeschl-Wilson et al., 2008), the reductions in pasture contamination (from reduced FEC) potentially lead to substantially increased performance (Bishop and Stear, 1997, 1999; Laurenson et al., 2012). Various experimental studies now support these theoretical predictions of epidemiological benefits arising from populations of animals excreting fewer eggs (Gruner et al., 2002; Leathwick et al., 2002; Williams et al., 2010).

A potential down side of selection for increased resistance is the possibility of evolution of the nematode population, analogous to the evolution of anthelmintic resistance in response to indiscriminate use of anthelmintics. This topic has been considered in detail by Kemper et al. (2009, 2010b), Kemper (2010) and summarized by Bishop (2012). Briefly, experimental evidence has failed to show that nematodes adapt differentially to resistant and susceptible hosts, at least as far as the experimental system had power to detect such effects (Kemper et al., 2009).

Secondly, modeling studies have suggested that the advantages of resistant hosts in terms of reduced FEC should be maintained for many host generations. These results are partially due to the highly polygenic nature of variation in resistance (Kemper et al., 2011), and the expected slow rates of parasite evolution are in stark contrast to those expected for anthelmintic resistance.

With the exception of FEC and growth rate under parasitized conditions, which is self-evidently a selection criterion in nearly all sheep breeding programs, of the other selection criteria mooted above, it appears only to be selection for decreased treatment requirements (an indirect indicator of resilience) that has been implemented. Indeed, Morris et al. (2010) found that long-term selection for decreased treatment requirements was effective, albeit complex to implement, leading to decreased breech soiling, and increased growth rate whilst not altering resistance. Although the other immunological or metabolic traits are invariably heritable and genetically correlated with nematode resistance traits, I am not aware of long-term selection performed on such traits in ruminants. However, it may be wise to exercise caution before advocating selection on indicator traits before their time- and challenge-dependent properties are known. For example, Davies (2006) estimated genetic correlations of indicator traits such as IgA or eosinophil concentrations with FEC or worm fecundity across different ages. Not only did she find that the correlations changed over time, but they often changed sign between times when lambs presumably had immature immune responses (e.g., immediately post-weaning at 3 months of age) and when they had more mature immune responses (e.g., 6 months). Therefore, the age and exposure history of animals must be clearly defined before selecting on traits that change with increasing exposure.

CONSIDERATION OF TOLERANCE

To date, the discussion has avoided the concept of tolerance, i.e., the decline in performance as infection level increases. At the breed level, genetic differences in impacts of infection in sheep, presumed to be tolerance, have been clearly and elegantly demonstrated in the comparison of Red Maasai and Dorper sheep, in environments differing in level of challenge (Baker et al., 2004). In fact, this was interpreted as a genotype by environment interaction, as described above in section "Estimation of Tolerance." The Red Maasai breed is considered to be relatively resistant and is termed resilient, on account of its PCV levels following exposure to *H. contortus* (Baker et al., 2003). The same authors observed the Dorper breed to be considerably more susceptible and less resilient to *H. contortus*. Productivity of these two breeds varies considerably according to environment, with Red Maasai sheep being more efficient than Dorper sheep in a high challenge environment, whereas in a low challenge semi-arid environment there were negligible breed differences in productive efficiency (Baker et al., 2004). Even after accounting for differences in resistance, this equates to the Red Maasai breed being more tolerant of infection than the Dorper breed.

In principle, measurement of tolerance at the breed or group level is relatively straightforward for nematode infections, as the measurements required to estimate tolerance (i.e., performance and FEC at different levels of challenge) are readily available [see Kause (2011) and Doeschl-Wilson et al. (2012a), this volume]. At a level down from the group level, there may also be possibilities to assess tolerance at the sire family level. Large datasets that are now becoming available as a result of industry- or breed-wide breeding programs may provide the data to allow this, provided that nematode resistance traits (i.e., FEC) have been measured alongside performance traits. Using data where sires have been evaluated across years and across farms differing in infection level may permit the estimation of tolerance at the sire level. If this succeeds, it may enable customization of breeding objectives by environment, with (sire) tolerance given greater weight in environments that provide greater parasite challenges.

Measurement of tolerance at the individual animal is, as described above, considerably more difficult. Although the major impact of nematode parasitism is on growing lambs, it will almost certainly not be possible to assess performance on the same lamb at different levels of challenge, as challenge level and exposure-dependent acquisition of immunity will be confounded. However, in principle, individual animal tolerance can be assessed in traits expressed by adult animals. The adult ewe is generally only affected by nematode infections during the periparturient period, when the impacts of late gestation and early lactation lead to a temporary waning of immunity (Taylor, 1935). During this period, ewe "productivity" may be defined as milk production, which is reflected in the growth rate of her lambs. Genetic correlations between ewe FEC and the growth rate of her lambs during this period have been reported by Bishop and Stear (2001); these were positive suggesting a nutrient partitioning or resource allocation effect, i.e., ewes preferentially allocating resources to lactation instead of immunity tended to have lambs which grew faster simultaneously with a higher FEC (and vice versa). Whilst this suggests an impact of nematode infections on performance it does not directly give information on tolerance. However, datasets such as this, with repeated observations across years on both the infection trait (FEC) and the performance trait (lamb growth), do potentially allow estimates to be made of individual animal tolerance, regressing performance on FEC. Once estimated, this will allow exploration of the genetic properties of individual animal tolerance.

A ROLE FOR GENOMICS

This Review so far has been mainly concerned about trait definition, i.e., the phenotypic side of genetic improvement. However, genomics may have an added-value role to play in the optimal selection for resistance/tolerance in relation to nematode infections. Genomic selection, based on concepts outlined by Haley and Visscher (1998) and Meuwissen et al. (2001) is now well-established in the dairy cattle sector. In principle, it could also be applied to the small ruminant sector although this would require a marked change in available genomic tools as small ruminants do not have the advantages seen in the dairy sector of high animal value, small effective population size, and highly accurate phenotypes (i.e., daughter trait averages) to calibrate the predictions. Further, published studies of genomic prediction of nematode resistance suggest only moderate accuracy with currently available SNP arrays (Kemper et al., 2011). However, under the assumption

that more powerful genomic tools come available, the concept is worth pursuing.

The key advantage of genomic selection is that, once calibrated, it can reduce the requirement for intensive ongoing trait recording. For a trait as inherently difficult to measure as nematode tolerance, this could be advantageous. As described above, and also by Doeschl-Wilson et al. (2012a,b) tolerance is readily assessed at the group or sire family level. A trait defined at the sire family level is analogous to sex-limited traits seen in dairy cattle, such as milk production, where the EBV is estimated from progeny performance. In these circumstances genomic predictions are readily made from de-regressed estimated breeding values. Similarly in sheep, sires with progeny in high and low nematode challenge environments enable, in principle, EBVs for tolerance to be estimated for the sire, and hence genomic predictions of tolerance for next-generation animals to be made using SNP arrays. Therefore, genomics may allow prediction of individual animal tolerance to be made in situations where individual animal phenotypes are difficult to obtain.

As described above, this is an "in principle" use of genomics to help address tolerance of nematode infections in sheep. Making this work in practice would require large datasets on performance and infection levels for genetically related animals in different environments, and probably cheaper yet more powerful genomic tools than available at the time of writing.

CONCLUSIONS

In conclusion, whilst tolerance is an appealing concept, and one that is much discussed when considering disease resistance, and also debated in ecological and evolutionary discussions, it is actually a difficult trait to use in practical situations. In particular, the complexity of measuring individual animal tolerance makes it difficult to implement into breeding programs, although novel analytical solutions to this problem are proposed in this volume (Doeschl-Wilson et al., 2012b). In many cases, geneticists believe they are measuring tolerance when in actual fact they are looking at a composite trait combining tolerance and resistance. Further, the utility of tolerance as a breeding goal depends on the epidemiology of the disease, as it is only useful when infection is prevalent.

Conceptually, the utility of tolerance in a breeding goal, assuming that it can be measured, depends on many factors, including animal genotypes for resistance, for tolerance, for productivity under situations of no challenge and the covariance amongst these traits. Further, because of the dependence of tolerance on the prevalence of infection, factors which influence prevalence also become important. This includes population mean resistance, especially the infectivity component of resistance, as this will influence the force of infection faced by the whole population.

In principle tolerance is applicable to nematode infections, as these infections usually lead to a prevalence approaching unity and nematode infections are not zoonotic. However, even in this situation tolerance is hard to estimate: it may be estimated at the breed or sire family level, but rarely can it be estimated at the individual animal level. An exception may be for nematode infections in lactating animals, because in this case data from separate lactations on the same animal may be considered as independent expressions of the same trait, with different infection levels in different years.

Whilst it may not be possible to obtain unbiased estimates of tolerance for most traits, various pragmatic solutions may capture the information necessary to design effective breeding programs. For example, by measuring resistance (FEC) and performance in a parasitized environment (sometimes referred to as resilience, see **Box 1**), sufficient information is available to improve both performance and resistance in that environment, with improvements in resistance leading to further indirect benefits via decreased pasture contamination. Accounting for the tolerance component of environmental sensitivity (hence genotype by environment interactions) would require information from farms varying in degree of nematode challenge, with these farms linked by usage of common sires. Such information will already be available in many structured breeding programs, enabling estimation of genotype by environment interactions, and determination of whether breeding goals customized by (parasite) environment are necessary. Therefore, in practice it may be possible to capture the benefits of tolerance to nematode infections for livestock, without necessarily having to obtain unbiased estimates of this trait.

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REFERENCES

Anderson, R. M., and May, R. M. (1991). *Infectious Diseases of Humans. Dynamics and Control.* Oxford: Oxford University Press.

Baker, R. L., Mugambi, J. M., Audho, J. O., Carles, A. B., and Thorpe, W. (2004). Genotype by environment interactions for productivity and resistance to gastro-intestinal nematode parasites in Red Maasai and Dorper sheep. *Anim. Sci.* 79, 343–353.

Baker, R. L., Nagda, S., Rodriguez-Zas, S. L., Southey, B. R., Audho, J. O., Aduda, E. O., et al. (2003). Resistance and resilience to gastrointestinal nematode parasites and relationships with productivity of Red Maasai, Dorper and Red Maasai X Dorper crossbred lambs in the sub-humid tropics. *Anim. Sci.* 76, 119–136.

Bishop, S. C. (2012). Possibilities to breed for resistance to nematode parasite infections in small ruminants in tropical production systems. *Animal* 6, 741–747.

Bishop, S. C., and Morris, C. A. (2007). Genetics of disease resistance in sheep and goats. *Small Rumin. Res.* 70, 48–59.

Bishop, S. C., and Stear, M. J. (1997). Modelling responses to selection for resistance to gastrointestinal parasites in sheep. *Anim. Sci.* 64, 469–478.

Bishop, S. C., and Stear, M. J. (1999). Genetic and epidemiological relationships between productivity and disease resistance: gastrointestinal parasite infection in growing lambs. *Anim. Sci.* 69, 515–525.

Bishop, S. C., and Stear, M. J. (2000). The use of a gamma-type function to assess the relationships of the between the number of adult *Teladorsagia circumcincta* and total egg output. *Parasitology* 121, 435–440.

Bishop, S. C., and Stear, M. J. (2001). Inheritance of, and factors affecting, egg counts during early lactation in Scottish Blackface ewes facing mixed, natural nematode infections. *Anim. Sci.* 73, 389–395.

Bishop, S. C., and Stear, M. J. (2003). Modeling of host genetics and resistance to infectious diseases: understanding and controlling nematode infections. *Vet. Parasitol.* 115, 147–166.

Bishop, S. C., and Woolliams, J. A. (2010). On the genetic interpretation of disease data. *PLoS ONE* 5:e8940. doi: 10.1371/journal.pone.0008940

Boddicker, N., Waide, E. H., Rowland, R. R. R., Lunney, J. K., Garrick, D. J., Reecy, J. M., et al. (2012). Evidence for a major QTL associated with host response to porcine reproductive and respiratory syndrome virus challenge. *J. Anim. Sci.* 90, 1733–1746.

Carval, D., and Ferriere, R. (2010). A unified model for the coevolution of resistance, tolerance, and virulence. *Evolution* 64, 2988–3009.

Davies, G. (2006). *Investigating Genetic Aspects of the Variation in the Host Response to Gastrointestinal Parasites in Sheep*. Ph.D. thesis, University of Glasgow.

Davies, G., Genini, S., Bishop, S. C., and Giuffra, E. (2009). An assessment of the opportunities to dissect host genetic variation in resistance to infectious diseases in livestock. *Animal* 3, 415–436.

Davies, G., Stear, M. J., and Bishop, S. C. (2005). Genetic relationships between indicator traits and nematode parasite infection levels in 6 month old lambs. *Anim. Sci.* 80, 143–150.

Doeschl-Wilson, A. B., Kyriazakis, I., and Villanueva, B. (2012a). The first step towards genetic selection for host tolerance to infectious pathogens: obtaining the tolerance phenotype through group estimates. *Front. Gene.* 3:265. doi: 10.3389/fgene.2012.00265

Doeschl-Wilson, A. B., Bishop, S. C., Kyriazakis, I., and Villanueva, B. (2012b). Novel methods for quantifying individual host response to infectious pathogens for genetic analyses. *Front. Gene.* 3:266. doi: 10.3389/fgene.2012.00266

Doeschl-Wilson, A. B., Vagenas, D., Kyriazakis, I., and Bishop, S. C. (2008). Challenging the assumptions underlying genetic variation in host nematode resistance. *Genet. Sel. Evol.* 40, 241–264.

Gandon, S., MacKinnon, M. J., Nee, S., and Read, A. F. (2001). Imperfect vaccines and the evolution of pathogen virulence. *Nature* 414, 751–756.

Gruner, L., Cortet, J., Sauve, C., Limouzin, C., and Brunel, J. C. (2002). Evolution of nematode community in grazing sheep selected for resistance and susceptibility to *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*: a 4-year experiment. *Vet. Parasitol.* 109, 277–291.

Haley, C. S., and Visscher, P. M. (1998). Strategies to utilize marker-QTL associations. *J. Dairy Sci.* 81, 85–97.

Jackson, F., and Coop, R. L. (2000). The development of anthelmintic resistance in sheep nematodes. *Parasitology* 120, S95-S107.

Karlsson, L. J. E., and Greeff, J. C. (2006). Selection response in faecal worm egg counts in the Rylington Merino parasite resistant flock. *Aust. J. Exp. Agric.* 46, 809–811.

Kause, A. (2011). Genetic analysis of tolerance to infections using random regressions, a simulation study. *Genet. Res.* 93, 291–302.

Kemper, K. E. (2010). *The Implications for the Host-Parasite Relationship When Sheep are Bred for Enhanced Resistance to Gastrointestinal Nematodes*. Ph.D. thesis, University of Melbourne.

Kemper, K. E., Elwin, R. L., Bishop, S. C., Goddard, M. E., and Woolaston, R. R. (2009). Adaptation of nematodes to 30 generations of serial passage through resistance or susceptible sheep. *Int. J. Parasitol.* 39, 607–614.

Kemper, K. E., Emery, D. L., Bishop, S. C., Oddy, H., Hayes, B. J., Dominik, S., et al. (2011). The distribution of SNP marker effects for faecal worm egg count in sheep, and the feasibility of using these markers to predict genetic merit for resistance to worm infections. *Genet. Res.* 93, 203–219.

Kemper, K. E., Palmer, D. G., Liu, S. M., Greeff. J. C., Bishop, S. C., and Karlsson, J. L. E. (2010a). Reduction of faecal worm egg count, worm numbers and worm fecundity in sheep selected for worm resistance following artificial infection with *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*. *Vet. Parasitol.* 171, 238–246.

Kemper, K. E., Goddard, M. E., and Bishop, S. C. (2010b). "A simple model of worm adaptation to livestock bred for improved worm resistance," in *Proceedings of the 9th World Congress on Genetics Applied to Livestock Production*, (Leipzig), 0164.

Laurenson, Y. C. S. M., Kyriazakis, I., Forbes, A. B., and Bishop, S. C. (2012). Exploration of the epidemiological consequences of host resistance to gastro-intestinal parasitism and grazing management of sheep through a mathematical model. *Vet. Parasitol.* 198, 238–249.

Leathwick, D. M., Atkinson, D. S., Miller, C. M., Brown, A. E., and Sutherland, I. A. (2002). "Benefits of reduced larval challenge through breeding for low faecal egg count in sheep. Novel Approaches, III," in *A Workshop Meeting on Helminth Control in Livestock in the New Millennium*, (Midlothian: Moredun Research Institute).

Meuwissen, T. H. E., Hayes, B. J., and Goddard, M. E. (2001). Prediction of total genetic value using genome-wide dense marker maps. *Genetics* 157, 1819–1829.

Morris, C. A., Bisset, S. A., Vlassoff, A., Wheeler, M., West, C. J., Devantier, B. P., et al. (2010). Selecting for resilience in Romney sheep under nematode parasite challenge, 1994–2007. *N.Z. J. Agric. Res.* 53, 245–261.

Morris, C. A., Wheeler, M., Watson, T. G., Hosking, B. C., and Leathwick, D. M. (2005). Direct and correlated responses to selection for high or low faecal nematode egg count in Perendale sheep. *N.Z. J. Agric. Res.* 48, 1–10.

Perry, B. D., McDermott, J. J., Randolph, T. F., Sones, K. R., and Thornton, P. K. (2002). *Investing in Animal Health Research to Alleviate Poverty*. Nairobi, Kenya: International Livestock Research Institute (ILRI).

Råberg, L., Graham, A. L., and Read, A. F. (2009). Decomposing health: tolerance and resistance to parasites in animals. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 364, 37–49.

Råberg, L., Sim, D., and Read, A. F. (2007). Disentangling genetic variation for resistance and tolerance to infectious diseases in animals. *Science* 318, 812–814.

Roy, B. A., and Kirchner, J. W. (2000). Evolutionary dynamics of pathogen resistance and tolerance. *Evolution* 54, 51–63.

Simms, E. L. (2000). Defining tolerance as a norm of reaction. *Evol. Ecol.* 14, 563–570.

Stear, M. J., Bishop, S. C., Bairden, K., Duncan, J. L., Gettinby, G., Holmes, P. H., et al. (1997). How hosts control worms. *Nature* 389, 27.

Taylor, E. L. (1935). Seasonal fluctuation in the numbers of eggs of Trichostrongylid worms in the faeces of ewes. *J. Parasitol.* 21, 175–179.

Vagenas, D., Doeschl-Wilson, A., Bishop, S. C., and Kyriazakis, I. (2007). Exploration of the effects of host genotype and nutrition on the genetic parameters of lambs challenged with gastrointestinal parasites. *Int. J. Parasitol.* 37, 1617–1630.

Vagenas, D., Jackson, F., Russel, A. J. F., Merchant, M., Wright, I. A., and Bishop, S. C. (2002). Genetic control of resistance to gastrointestinal parasites in crossbred cashmere producing goats: responses to selection, genetic parameters and relationships with production traits. *Anim. Sci.* 74, 199–208.

Waller, P. J. (1997). Anthelmintic resistance. *Vet. Parasitol.* 72, 391–405.

Williams, A. R., Greeff, J. C., Vercoe, P. E., Dobson, R. J., and Karlsson, L. J. E. (2010). Merino ewes bred for parasite resistance reduce larval contamination during the peri-parturient period. *Animal* 4, 122–127.

Woolaston, R. R., and Piper, L. R. (1996). Selection of Merino sheep for resistance to *Haemonchus contortus*: Genetic variation. *Anim. Sci.* 62, 451–460.

Woolaston, R. R., and Windon, R. G. (2001). Selection of sheep for response to *Trichostrongylus colubriformis* larvae: genetic parameters. *Anim. Sci.* 73, 41–48.

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Selection of pigs for improved coping with health and environmental challenges: breeding for resistance or tolerance?

Sarita Z. Y. Guy[1]*, Peter C. Thomson[1] and Susanne Hermesch[2]

[1] *ReproGen Animal BioScience Group, Faculty of Veterinary Science, University of Sydney, Sydney, NSW, Australia*
[2] *Animal Genetics and Breeding Unit, a joint venture between University of New England and NSW Department of Primary Industries, University of New England, Armidale, NSW, Australia*

Edited by:
Ilias Kyriazakis, Newcastle University, UK

Reviewed by:
Ilias Kyriazakis, Newcastle University, UK
Luca Fontanesi, University of Bologna, Italy

****Correspondence:***
Sarita Z. Y. Guy, Faculty of Veterinary Science, Room 505, RMC Gunn Building (B19), University of Sydney, NSW 2006, Australia.
e-mail: sarita.guy@sydney.edu.au

The benefits of improved health and welfare in pigs have driven refinements in management and selection practices, one of which is the production of pig phenotypes that can maintain health and productivity by improving response against pathogens. Selection has traditionally been made for host resistance; but the alternative host defence mechanism—host tolerance—is now being considered, as breeding for disease tolerance allows maintenance of high performance across environments of increasing pathogenic load. A distinction must be made between these two mechanisms as they vary in their influence on host-pathogen interactions and pathogen evolution, and consequently on the results of breeding programs. Many pig production studies have failed to distinguish between resistance and tolerance; although a distinction may not always be possible. This article reviews current perspectives in selective breeding for disease resistance and tolerance in growing pigs, and the attendant industry implications. To assess the viability of breeding for resistance and/or tolerance for improved response to disease and other environmental challenges, we propose the use of routine farm records, instead of data measurements taken from laboratory experiments. Consequently, a number of factors need to be taken into account simultaneously for a multidimensional modeling approach. This includes not only genotype and disease variables, but also descriptors of the environment, as well as any possible interactions. It may not be feasible to record individual pathogen loads, and therefore true tolerance, on farm using routinely collected data. However, it may be estimated with group (farm) means, or other proxy measures. Although this results in a bias, this may still be useful for modeling and quantifying resistance and tolerance. We can then quantify success of selection, and this may enable us to decide whether to select for disease resistance versus disease tolerance.

Keywords: host defence strategies, reaction norm, resistance, tolerance, pig breeding

INTRODUCTION

The increase in societal pressure for sustainable pork production that incorporates optimum health and welfare highlights the need for alternative, more holistic approaches in genetic selection programs (Kanis et al., 2005; Knap, 2012; Merks et al., 2012). The long-term focus of pig breeding programs worldwide has traditionally been for high productivity. This has resulted in an increase in behavioral, physiological, and immunological problems, greater susceptibility to stress and disease (Rauw et al., 1998; Prunier et al., 2010), and an increasing difficulty for the highly productive pigs to cope with environmental challenges (Schinckel et al., 1999).

The environment of the pig may be a determinant of disease manifestation, and although its control to meet pig requirements improves production and reduces stress (Black et al., 2001), it may neither be economically feasible nor necessarily possible in all circumstances to control environmental conditions (Kerr and Hines, 2005). For example, pigs selected in high health environments, usually observed in nucleus herds, may not perform as well in the more challenging environments possibly observed on commercial farms. Clearly an absence of genotype-by-environment interaction is preferred so that animals would remain healthy across varying environments and pathogenic challenges. One way of maintaining health is to build host defence mechanisms against challenges, the two strategies being resistance and tolerance (Doeschl-Wilson and Kyriazakis, 2012, this issue).

There have been several recent reviews comparing disease resistance and tolerance in the plant or ecological literature (Baucom and de Roode, 2011; Detilleux, 2011). The epidemiological consequences of breeding for disease tolerance in livestock have been briefly discussed by Bishop et al. (2002), although disease resistance was the main focus of the discussion. More recently, Råberg et al. (2009) discussed the implications of disease tolerance in animals, although the examples used

were predominantly based on mouse populations in laboratory conditions. However, these authors also highlight the usefulness of defining disease tolerance as a reaction norm for animal breeding applications, as has been done in plant breeding. A reaction norm quantifies the response of a genotype to varying environmental conditions, and variation in pathogen load is commonly used in reaction norm models to quantify disease tolerance of different genotypes.

The primary aim of this article is to discuss and disentangle the mechanisms of resistance and tolerance to disease and environmental challenges, with specific reference to pig production and its practical application. These two host defence strategies are distinguished by consequences of selection and of host-pathogen co-evolution, immunological mechanisms, and physiological measures. This review also assesses the use of routinely collected on farm records as possible variables and data structures to quantify resistance and tolerance. A general framework to model the relationship between these variables and possible outcome measures is also described. Selection for pigs that perform in a wide range of environments should incorporate not only ability to cope with pathogenic challenge(s), but also any environmental perturbations, which are often omitted in the modeling and prediction of resistance and tolerance.

DEFINING RESISTANCE AND TOLERANCE

Disease resistance can be defined as the active reduction of the pathogen burden or prevalence by inhibiting infection and reducing pathogen growth rate (Best et al., 2008). In pig breeding, the term disease resistance has been generally used when aspects of genetic improvement of the health status of pigs have been discussed (Rothschild, 1998; Doeschl-Wilson et al., 2009). The genetic control of disease susceptibility in pigs against the bacteria *Escherichia coli* is an example of disease resistance. A single allele is responsible for adhesion factors receptors in the host gut, which allows binding and infection of various *E. coli* strains (Gibbons et al., 1977). A homozygous recessive pig lacking these receptors avoids binding of the bacteria and is therefore a disease resistant animal (Gibbons et al., 1977).

Tolerance can be defined as a host's ability to limit the detrimental impact caused by a pathogen by counteracting the damage (Råberg et al., 2007; Read et al., 2008; Schneider and Ayres, 2008; Rohr et al., 2010). A tolerant host will therefore be more able to maintain productivity than a non-tolerant host, despite increasing pathogenic burden. The first example the authors are aware of that recognizes genetic differences in disease tolerance in animal breeding is by Atkins and Mortimer (1989), who used reaction norms to find differences in the response to varying incidence of fleece rot and body strike in sheep flocks. The genetic differences in tolerance in pigs were demonstrated by Potter et al. (2012) when average daily gain declined more strongly with increasing viral serum levels for purebred Duroc than synthetic White Pietrain pigs, although it was not termed as "tolerance."

It should be noted that in the ecological literature, the response of a resistant and/or tolerant individual is described as fitness and survival (Baucom and de Roode, 2011), whilst in an animal production context the response can also include productivity and health. It is important to recognize this as the inclusion of breeding for tolerance must also be economically viable, with improved productivity being the ultimate aim. This leads us to the distinction between terms tolerance and resilience, the latter being defined by Albers et al. (1987) as the "ability to maintain a relatively undepressed productivity level when infected." The term resilience usually conflates the two mechanisms of host defence, resistance, and tolerance. Depression in live weight gain due to infection was used by Albers et al. (1987) to measure disease resilience. Bisset and Morris (1996) point out that disease resilience defined in this way based on measurements available on farm may make use of the mechanisms of both resistance and tolerance. Breeding for resilience to nematode infections has been explored in sheep (Albers et al., 1987; Bisset and Morris, 1996; Gray, 1997). The inclusion of resilience in a productivity index was trialled with six New Zealand ram breeders, and although progress was slow due to low heritability, it was found to be practical and feasible (Morris et al., 2004). Recently, Morris et al. (2010) showed that selection for more resilient lines can delay the time until first drench, increase live weight at six months, and decrease breech soiling. These results demonstrate that it is possible to select for both productivity and improved health status by possibly making use of the mechanisms of both resistance and tolerance.

DISEASE TOLERANCE: THE DIFFERENCE TO DISEASE RESISTANCE

From all that has been written on the concepts of resistance and tolerance, it can be concluded that the distinguishing factor between the two is the interaction, or lack of, between host and pathogen. Unlike resistance, disease tolerance mechanisms do not directly affect the pathogen. However, it may not always be possible to make a clear distinction between the two mechanisms. For example, Lewis et al. (2007) review the genetic aspects of host responses to porcine reproductive and respiratory syndrome (PRRS). Although the authors acknowledge that there is a difference between resistance and tolerance, the responses reviewed were not specifically attributed to either of the two mechanisms. The phrasing "resistance or tolerance" indicates they were not able to distinguish between the two mechanisms.

INFLUENCES ON HOST-PATHOGEN INTERACTIONS (CO-EVOLUTION)

Pathogen evolution can counteract the attempts to control infectious disease using genetic management strategies, but only the relative, and not absolute, risk of this occurring can be calculated (Bishop and Mackenzie, 2003). Both mechanisms vary in influences on pathogen prevalence and fitness, creating different feedback systems and different evolutionary outcomes that may affect the ultimate success of a breeding program.

Selection for resistance can be seen as a negative feedback system on resistant-allele frequency in a population, as the reduction in pathogen prevalence also reduces the fitness advantage of carrying resistance alleles (Miller et al., 2005; Råberg et al., 2007). The loss in fitness advantage may limit the success of selection for resistance, and simulations have shown that selection for resistance results in polymorphisms instead of fixation of resistant alleles in the host (Roy and Kirchner, 2000; Miller et al., 2005;

Best et al., 2008). It can also be argued that mechanisms of host resistance exert a selective pressure on the pathogen, resulting in an increase in virulence (Svensson and Råberg, 2010). However, Bishop and Mackenzie (2003) note that the risk of pathogens evolving in response to this selective pressure can be reduced if more than one resistance gene is selected for. Other trade-offs between pathogenic responses to host resistance include other aspects of survival, which was demonstrated by Kemper et al. (2010); minimal survival outside the host resulted in fixation of the pathogen survival allele in a resistant host, whilst a large survival rate outside the host resulted in loss of pathogenic resistance to host resistance.

Alternatively, selection for tolerance imposes a positive feedback system, since the lack of impact on the pathogen may increase pathogen prevalence and therefore place additional selective pressure on tolerance alleles (Roy and Kirchner, 2000; Miller et al., 2005). The fitness advantage of tolerant genes increases with incidence of infection, driving tolerance alleles to fixation (Roy and Kirchner, 2000). Also, since there is no direct effect on the pathogen and therefore no direct selective pressure, a commensalism relationship between host and pathogen may be the outcome, instead of an antagonistic co-evolution (Miller et al., 2006). The pathogen benefits, but the host is neither harmed nor benefited, provided the host can tolerate the pathogen damage up to a certain level of pathogen load (Miller et al., 2006).

Although a tolerant population may result in commensal co-evolution between host and pathogen, integrating tolerance into a breeding objective has an element of difficulty due to possible consequences on herd health. Since there is no adverse effect on the pathogen, selection for tolerance allows animals to be a source of infection for susceptible animals and may result in an increase in transmission of infection. Breeding for tolerant pigs should therefore be part of a so called integrated health herd program (Lewis et al., 2007), which may initially control pathogen load. Such a program may also encompass control of other environmental factors, such as air quality, climatic conditions in sheds, and other husbandry measures. This approach should be employed not only on one farm, but across an entire industry (Lewis et al., 2007), with appropriate surveillance program, such as abattoir health monitoring.

IMMUNOLOGICAL MECHANISMS

The most direct approach to selecting for improved health of pigs is observation and selection of breeding stock according to disease status (Rothschild, 1998). However, a pig may be infected by a pathogen but may not always display clinical disease. An indirect indicator for disease incidence or animal health status is measurement of immune responsiveness. Immunological traits have been found to be associated with performance (Clapperton et al., 2008, 2009). Immunological traits have also been found to display genetic variation, within and between breeds (Henryon et al., 2006; Clapperton et al., 2009; Flori et al., 2011), demonstrating the possibility of breeding for resistance, tolerance, or both, through selection of an immune response. Mallard et al. (1992) challenged pigs with Hen Egg White Lysozyme (HEWL), synthetic peptide TGAL and sheep erythrocytes, and selected according to antibody and cell-mediated response (adaptive immunity), and monocyte function (innate immunity) of Yorkshire pigs. The heritability of these immunological traits ranged from 0 for monocyte function to 0.25 for secondary antibody response to HEWL. After eight years of selection, two distinct lines were formed: a high immune response (HIR) and low immune response (LIR).

This selection experiment also demonstrates that selection for response against a specific pathogen may have unfavorable consequences for other traits. After eight generations of selection, the HIR line had a higher incidence of arthritis after *Mycoplasma hyorhinis* challenge (Wilkie and Mallard, 1999). Furthermore, selection for response against one specific pathogen may have unpredictable effects to the response against other pathogens. Therefore, selection criteria and possible consequences of selection strategies should be assessed thoroughly before incorporation into a breeding program. Improving the understanding of specific immune functions in the distinct mechanisms of disease resistance versus disease tolerance will hopefully help avoid unfavorable correlated responses.

It should also be noted that different immune responses (including innate, cellular, and humoral) are produced for different pathogens, and higher levels of immune responses may not always lead to or indicate improved resistance (Adamo, 2004). Many studies assume that a low immunological response corresponds to a lower disease resistance, which may not necessarily be true. This is because the correlations between assays of immunity and disease resistance may be weak and pathogen-specific (Adamo, 2004). Different types of pathogens may elicit a different strength of response varying in time, space, and type. The variable immune response of the pig to different pathogenic challenges was highlighted by Salak-Johnson and McGlone (2007). Therefore, the type of immune response should be analysed critically before attempting to measure resistance and/or tolerance.

Bishop and Woolliams (2004) proposed pig genetic improvement by means of increasing "generalized immunity" to respond effectively to pathogenic challenge, i.e., promotion of the innate immune system. This "immune robustness", as termed by Kaiser (2010), allows improved performance, health and welfare by reducing the impact of subclinical disease. Neither of these authors discusses whether a general or robust immunity will be beneficial for maintaining health and productivity across varying infection levels. Genetic improvement of disease tolerance implies that a genotype by infection level interaction exists for performance, health, or immune traits. Genotype by PRRS infection level interaction for reproductive traits was demonstrated by Lewis et al. (2009). Additional information about potential genotype by health status interactions has been reported by Clapperton et al. (2008) and Clapperton et al. (2009), who found different heritability estimates for pig herds with different health status for some immune traits. Heritabilities were higher in high health status for CD4+ and CD11R1+ cells in both studies. Estimates ranged from 0.32 to 0.82 in the high health, and from 0.07 to 0.57 in the low health environments for these two traits. However, the higher heritability estimates of 0.37 ($\pm$0.16) for white blood cell counts and of 0.69 ($\pm$0.21) for B cells in lower health status presented by Clapperton et al. (2008), were not observed in SPF pigs in the subsequent study by Clapperton et al.

(2009). These heritability estimates had varying levels of precision, with estimates of standard errors ranging from 0.09 to 0.22. Therefore, sampling effects may have contributed to the discrepancies in estimates of heritabilities and further large-scale studies are required to determine whether genotype by infection level interactions exist for immune traits.

Many studies have not been able to distinguish between the immune response for disease resistance and tolerance. For example, the Lewis et al. (2007) review identified immunological mechanisms of host response to PRRS, but the authors were not able to conclude whether the immune responses were responsible for virus resistance (eradicating the virus from the host) or tolerance (negating the effects of virus damage). The deficiency of information on the specific immunological responses related to tolerance questions the reliability in using these measurements in the quantification of and selection for resistance and tolerance.

With the pig genome characterized and available, it should be acknowledged that marker assisted selection and genomic selection can be powerful selection tools for traits that are difficult to measure. Further, new developments using molecular information can be used to better understand physiological traits, such as immune response to pathogen challenge. Lunney and Chen (2010) reviewed the quantitative trait loci (QTLs) and candidate genes for the immune response of disease resistance to PRRS. Genomic regions associated with other resistance and tolerance measures have also been identified. For example, Boddicker et al. (2012) found viral loads (estimated through blood samples) to have a heritability of 0.28, and have detected associations with the genomic regions on chromosomes 3, 4, and X. Weight gain had a heritability of 0.26 and was associated with regions on chromosomes 1, 4, 7, and 17 (Boddicker et al., 2012). Although the identification of the genes responsible for resistance is relevant, the purpose of this article is to discuss how to disentangle the mechanisms of resistance and tolerance. Most research has focused on resistance, without making a distinction from tolerance. In order to clarify if resistance and tolerance are simply different expressions of the same trait, or indeed are genetically different traits, we first need to estimate the genetic correlation (r_G) between these two traits, with different traits indicated by an r_G of less than one. Further indications of separate genetic control of these traits can be examined from QTL mapping or genome-wide association study (GWAS) approaches.

Traits that ameliorate the damage caused by the pathogen itself, or the damage caused by the host response (such as inflammation) need to be examined in order to quantify tolerance. Bergstrom et al. (2012) recently reviewed the innate host tolerance response to enteric bacteria, and verified that although resistance and tolerance responses both fight pathogenic challenges, tolerance mechanisms repair the damage caused by resistance mechanisms. The authors concluded that resistance and tolerance responses seem to complement each other.

To further understand mechanisms of tolerance, non-pathogenic interactions including non-reactivity to antigens such as intestinal flora, may be examined. Medzhitov et al. (2012) argue that general tolerance mechanisms should result in positive preconditioning, and tolerance mechanisms activated against one pathogen would increase tolerance to another unrelated pathogen. However, a selection program for disease tolerance without resistance may have consequences not only for herd health, as discussed in section "Influences in Host-pathogen Interactions," but also immunological consequences for the neonatal pig. Neonates are born immunologically naïve (Blecha, 1998), and selecting for tolerance and the possibility of an increase in transmission of infection may increase piglet mortality.

ENVIRONMENTAL CHALLENGES

Maintaining production when facing challenges is part of a host's phenotypic plasticity, specifically how individuals respond to their environment (Roff, 1997). With changes in consumer demand for welfare friendly pig production, there is a need to breed for genotypes that are less sensitive not only to pathogenic challenges but also other environmental challenges (Knap, 2005). These challenges include external stressors such as extremes in temperature, low-quality feed, or poor air quality. Although all of these challenges may have a significant influence on the performance of growing pigs (Black et al., 2001), environmental perturbations are usually not included in the evaluation of resistance and tolerance.

The role of stress in affecting the immune response and the possible interactions with social and environmental stressors for the pig were outlined by Salak-Johnson and McGlone (2007). Their review demonstrates that the indirect measure of health, and therefore resistance and tolerance, through immune responsiveness may not necessarily be independent of the environment. The lack of literature that includes environmental factors in the investigation of disease resistance and tolerance may reflect the assumption that these environmental factors are supposedly constant. However, when using data collected on farm, the environment of the pig may not always be constant. Also, any environmental challenges faced are important aspects of resistance and tolerance, especially since the effects of all perturbations are cumulative (Black et al., 2001). Therefore, we emphasize the inclusion of environmental challenges in models when investigating the mechanisms of disease resistance and tolerance.

Just as the immunological response varies according to class of pathogen, there are various physiological responses to environmental stress. They can include chemical/hormonal responses, as well as behavioral responses. An extreme example in pig production is the physiological response engendered by the alleles of the halothane gene. The halothane genes has been identified as a susceptibility gene that enhances occurrence of porcine stress syndrome, which results in pale, soft, and exudative (PSE) meat and affects multiple performance and carcass traits (Sather et al., 1991; Leach et al., 1996; Mérour et al., 2009). Other responses to physiological stress most commonly include chemicals and hormones such as cortisol. These physiological responses in pigs were reviewed by Kerr and Hines (2005), who introduced the term "stress resistance" which was used interchangeably with "stress tolerance," showing the two mechanisms have not really been distinguished and disentangled.

It should be acknowledged that the definition of pathogenic infection used in this article includes both micro- and macroparasites, and that disease manifestation may occur indirectly, such as

by means of ingestion of toxins, including mycotoxins produced by fungi. Since this can be considered as an environmental challenge, a measurement of toxin levels can therefore be included as a predictor variable in the quantification of disease resistance and tolerance.

QUANTITATIVE ANALYSIS

The focus of this section is definition and critique of the potential variables that may be appropriate for the modeling, quantification, and prediction of resistance and tolerance of pig genotypes. Although we provide examples of methodology and functions that may be utilized in pig breeding programs, this is a generic framework, and specifics depend on the set up of variables used. The techniques briefly described are not restricted to classical linear, but may include non-linear and/or non-normal relationships, as mentioned below. Further, they may be extended to mechanistic models (Bishop, 2010), which attempt to model the biological processes that could drive the outcome, rather than being a purely descriptive model. Regardless of the type of modeling undertaken, for optimal benefit to the pork industry, attempts should be made to exploit and be based on routine farm records, instead of the usual data measurements taken from laboratory experiments.

CONSTRUCTING A MODEL

Modeling has been proven to be a useful tool to better understand the complex interaction between host response and influencing factors, and to quantify the benefits of selection (Bishop, 2010). In the simplest case, models connect one or several outcome variables to a set of predictor variables according to some function, which may or may not be a simple function.

$$E(Y) = f(x_1, x_2, \ldots, x_p)$$

where Y is the response variable dependent on the p predictor variables $x_1, x_2, \ldots, x_p$.

There are several approaches to modeling such relationships, but they are generally based on the change of mean trait values as the host responds to challenges (Buehler et al., 2010). Statistical approaches in plant literature can be extended to the quantitative analysis of resistance and tolerance in animal production (Råberg et al., 2009). We will now consider the appropriate response and predictor variables for model specification.

The response variable

Resistance is typically measured as the inverse of pathogen burden and the response variable to quantify resistance is number of pathogens per host. For example, faecal egg count has been used in sheep breeding as a measure of resistance (Albers et al., 1987). Tolerance is defined as the slope of a regression of a host's response to variation in pathogen burden (Simms and Triplett, 1994; Råberg et al., 2009). The response variable to quantify tolerance may be based on performance measures, health status, and survival of pigs. For example, growth rate has been used as an indicator of health status of pig herds (Clapperton et al., 2009), which may decrease when pigs become infected, even when there are no visible signs of disease (i.e., subclinical disease).

The use of health disease status (yes, no) or clinical signs of disease infection (none, mild, severe) as a response variable may not be sufficiently accurate due to subclinical disease. For example Williams (1998) raised pigs in low-immune stimulation (vaccination) and high-immune stimulation (continuous flow of pigs and no injectable antibiotics) environments, and although both groups showed no clinical signs of disease, high-stimulation pigs consumed 5.5% less feed, grew 17% slower, produced 17% greater back fat, and 15% less eye muscle area.

Direct methods of measuring response to changing environments include challenging and then observing breeding stock, sibs, progeny, or clones of breeding stock after exposure to infectious challenge (Rothschild, 1998). Indirect indicators of health can include immunological and physiological responses. Reed and McGlone (2000) found that two PIC lines with similar immune status exposed to two distinct environments showed different immunological responses, indicating that immunological responses may be utilized for an indirect measure of response to change in environment. However, immunological measures should be used with caution as a higher response may not necessarily indicate a decline in performance or health, as discussed in section "Immunological Mechanisms."

Whether the response trait is labile or non-labile has important implications for a study. If looking at a non-labile trait (practically fixed during some period and not easily changeable), more observations across multiple individuals need to be used compared to when investigating a labile trait (an easily adjustable trait e.g., amount of voluntary feed intake). Since there would be greater variability expressed, it may be easier to exploit and select from a response variable that is labile.

The predictor variables

There are several sets of predictor variables to be considered when modeling resistance and tolerance. An obvious set is genotypes, commonly designated **g**. Such a set may comprise different breeds, sire lines, or other categories of families. The genotype set may also comprise of a single pig, if multiple measures are available for a pig that experiences varying environmental conditions. Further, this may be extended to include genomic information as trait predictors. At one level, marker information may be used for QTL mapping, and once these genomic regions are identified, a subset of markers can be used as a panel for marker-assisted selection. At the other end of the spectrum, complete genomic SNP information may be used to develop a genomic selection approach. Such strategies have been put forward for host responses to PRRS by Boddicker et al. (2012).

Another set of predictor variables, **d**, aims to describe the disease environment that genotypes may be exposed to. The key requirement to measure resistance and tolerance is variation in the disease environment. The ideal predictor variable to describe the pathogenic environment is pathogen load. A key issue is whether pathogen load is measured or can be measured in the environment, or in the host (level of infectivity). The use of environmental pathogen load is based on the assumption that infection across all animals occur at the same point in time, and does not allow for variation in immune responses by the host. Further, if the aim is to focus on input variables that are

readily available on farm and not on measures that are collected under experimental conditions, an indirect measure (or proxy) of pathogen load may need to be defined. For example, if a link between pathogen load and, level of medication, performance or survival rate is established, then these indirect measures may be used as a proxy for pathogen load. This approach was used by Lewis et al. (2009), who used on farm records of reproductive performance to identify when a PRRS infection occurred on farm.

Another issue is whether measures of individual pathogen load, as opposed to group estimates of pathogen load should be used. It may only be feasible on farm to measure groups. However, Doeschl-Wilson et al. (2012, this issue) argue that in order to obtain unbiased tolerance estimates, individual measures of pathogen load are required. Furthermore, many studies assume infection by a single pathogen type, when many hosts often harbor more than one pathogen, or pathogenic strains, simultaneously (Miller et al., 2006). Therefore, there may be more than one pathogenic burden to measure. Inclusion of pathogen load can include individual pathogen loads, or may be combined to form an overall pathogen load index.

As well as the disease environment, the response is also influenced by other non-disease environmental factors, **e**, and therefore one would also need to include any environmental perturbations when modeling response to selection for resistance and tolerance. These may include fluctuations in temperature, humidity, changes in social dynamics, air quality, stocking density, and changes in feed composition. Just as with pathogen load, on farm measures of non-disease environmental factors may only be feasible for groups of pigs and not individual pig. An overall pig farm health index, including health indicators, farm hygiene, and reproductive disturbances, can also be utilized to describe the environment, as proposed by Madec et al. (1993).

Therefore, the set of predictor variables may be partitioned into $\mathbf{x} = (\mathbf{g}, \mathbf{d}, \mathbf{e})$. Consequently, our generic model may be expressed as:

$$E(Y) = f(\mathbf{g}, \mathbf{d}, \mathbf{e})$$

MODELING THE FUNCTIONAL RELATIONSHIP, *f*

Having defined the response Y and predictor variables $\mathbf{x} = (\mathbf{g}, \mathbf{d}, \mathbf{e})$, these need to be connected by means of the function f, and we now discuss some general considerations.

Firstly, in order to assess tolerance across genotypes, interaction terms $\mathbf{g} \times \mathbf{d}$, $\mathbf{g} \times \mathbf{e}$, and possibly $\mathbf{d} \times \mathbf{e}$ and $\mathbf{g} \times \mathbf{d} \times \mathbf{e}$ need to be included in the model. In particular, it is the genotype by disease ($\mathbf{g} \times \mathbf{d}$) interaction(s) that quantify differences between genotypes in tolerance to pathogen load. In addition, it may be useful to quantify tolerance to environmental effects across different genotypes, hence the need to investigate $\mathbf{g} \times \mathbf{e}$ interactions, and possibly $\mathbf{g} \times \mathbf{d} \times \mathbf{e}$ interactions. Ignoring these interactions may lead to biased estimates of genetic differences in disease tolerance. All of these terms then might be specified as an additive model, which, in its simplest form, may be the usual linear regression model.

$$E(Y) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_p x_p,$$

using the x_i to include any of the above terms as well as their interactions.

It is possible that the total number of predictor variables contained in $\mathbf{x}$ may be quite large, and in some situations may even exceed the number of observations, n. This may happen, for example, when $\mathbf{g}$ includes genomic information. Although this cannot be handled in standard additive and generalized additive models (GAM), it can be addressed through the classical technique of reduction through use of principal component analysis or other techniques including partial least squares (Abdi, 2010).

Of course, further decisions on the form of f need to be made according to the class of response variable Y. If it is a continuous measure such as growth rate, one of the normal-distribution-based methods will be applicable, in the form of a linear, non-linear or perhaps spline model. However, if the response is binary, such as disease presence or absence, then a logistic regression model or extension to a GAM (Ruppert et al., 2003) would be appropriate. Further possibilities for instance, survival time, would require a Cox's proportional hazard model to be used, which again has the ability to include non-linear functions of predictor variables (see Cecchinato et al. (2008) for an example).

This then leads into considering the graphical interpretation of assessing tolerance and resistance. The simplest graphical representation of the interaction between genotype and disease load, and the approach taken by most tolerance studies, is a linear regression model. In animal breeding, this is commonly known as a reaction norm. Defining traits as functions through a reaction norm have been used to model the interaction between genotype and environment (Roff, 1997; Lynch and Walsh, 1998; Knap and Su, 2008; Kause, 2011). The reaction norm shows genotypic differences by the regression of phenotype against increasing pathogen burden of a single pathogen type, with separate slopes and intercepts for each genotype. For example, with only two genotypes, and for a normally distributed trait, the model might be expressed as:

$$E(Y) = \beta_0 + \beta_1 G + \beta_2 D + \beta_3 G \times D$$

where G is a 0–1 indicator variable for the genotype, and D is the measure of disease pathogen load.

A fully resistant host is one that successfully blocks pathogen entry or eliminates the pathogen, and there is no disease beyond an arbitrary threshold. A fully tolerant host is one whose phenotype/performance is not affected by the level of pathogen burden. A host can be tolerant and non-resistant, resistant and non-tolerant, or tolerance and resistant, shown as genotypes G1, G2, and G3, respectively, in **Figure 1**. It should be noted that this is an outline of the concept and the actual levels of performance or health of resistant versus tolerant pigs for a given pathogen burden will depend on the specifics of each situation. Whilst this representation is easily understandable, in reality, there may be non-linear responses between $E(Y)$ and the x_i, so that some of the linear terms may be replaced by polynomial or spline terms, allowing a more flexible approach to modeling non-linear relationships (Ruppert et al., 2003). Further, complex interactions

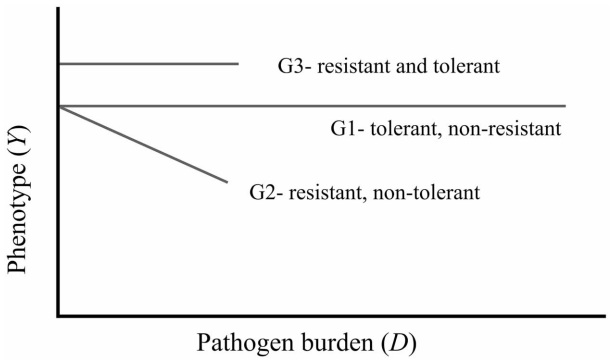

FIGURE 1 | The common portrayal of phenotypic responses of genotypes with a single predictor variable of pathogen burden. The three genotypes represented here are tolerant and not resistant (G1), resistant and not tolerant (G2), and resistant and tolerant (G3).

between two continuous predictors can be accommodated by the use of "thin plate spline" techniques. Råberg et al. (2009) discuss implications of non-linear reaction norms for disease tolerance, which may also arise from a genotype-by-environment interaction for the host's response to an unmeasured factor of the environment. The authors suggest conducting studies in homogenous environments, ideally in laboratories, to avoid any potential bias due to interactions of the genotypes and other unknown environmental factors. Clearly, this is not a solution for pig breeding applications, and any model quantifying disease tolerance needs to include as much detail as possible about other environmental factors.

Since there are multiple factors impacting on a host's ability to maintain production, this representation of resistance and tolerance is also too simplified. There needs to be a multivariable approach that will utilize known factors, of not only other pathogenic burdens, but also environmental variables that should not have an assumed linear relationship with the measured variable. The result would be a multidimensional model.

QUANTIFYING RESISTANCE AND TOLERANCE

Typically, definitions of resistance and tolerance are based on the linear model framework, as illustrated in **Figure 1**. With this, resistance can be defined quantitatively as the inverse of infection intensity (number of pathogens per host), while tolerance is indicated in the slope of the regression line (Simms and Triplett, 1994). That is, since the disease load in a resistant population is low (genotypes G2 and G3 in **Figure 1**), their inverse is high indicating resistance. In reality however, for this to be a useful metric, the external disease load in the environment should also be considered: to be resistant there must be indication that the load in the environment is considerably greater. Consequently it may be useful to quantify disease load relative to the load in the environment.

As discussed in the section "The Predictor Variables," it may not be feasible to obtain a true unbiased estimate of tolerance with on farm observational data due to the bias effects of group estimates (Doeschl-Wilson et al., 2012, this issue). In addition to differentiating host and environmental pathogen load, measures must be taken at the relevant time, during various levels of pathogenic challenge and/or no challenge (Doeschl-Wilson et al., 2012, this issue). This implies that we need repeated sampling on farm, but defining how often measures should be taken depends on the type of pathogen, and how quickly the pathogen loads change.

Using the quantitative definition of tolerance as the regression slope, typically negative, it is clear that small regression slopes indicate superior tolerance of a genotype to a disease challenge. In quantifying tolerance of genotypes that respond to disease load in a non-linear fashion, the average slope may be used. Alternatively, the area under the curve of the regression line may be used (Pilson, 2000). Otherwise, other metrics or proxies for production, such as growth rate and survival may be used to quantify resistance and tolerance. In addition, multiple measures of disease burden can be handled by the collective measure of all the partial regression slopes (if a linear model is used), or a collective measure of all the slopes, averaged over their respective disease loads (for a non-linear model).

However, it is important to note that tolerance (as mentioned previously) is not just a measure of sensitivity to disease burden (**d**), but to other environmental perturbations, such as ambient temperature. The above procedure can be extended to those variables (**e**) using exactly the same methods. Extending further, it would be possible to quantify tolerance in relation to **d**, **e** as well as **d** × **e**, incorporating the interactions with **g** to assess between-genotype differences.

The quantification of resistance may not simply be the inverse of infection intensity, especially when environmental variables, **e**, are also taken into account. Furthermore, the definition of resistant or non-resistant genotypes has not been clearly defined; for example, what is the maximum observable pathogen load before a genotype is considered non-resistant? There may not be a specific threshold but an arbitrary comparison with other genotypes.

CONCLUSION

Whilst most of the focus of research in animal breeding has been on resistance to pathogens, the difference to tolerance needs to be recognized due to consequences on pathogen-host interactions. The lack of knowledge on immunological and physiological response mechanisms for these two host defence strategies restricts our ability for quantification. For optimum benefit to the pork industry, we emphasize the use of routinely collected on-farm data to model and predict selection for resistance and tolerance. This means that a simple one-dimensional reaction norm, with pathogen burden as the only explanatory variable, cannot be used. A number of factors need to be taken into account simultaneously, including not only genotype and disease variables, but also descriptors of the environment, as well as any potential interactions. It may not be feasible to record true tolerance using routinely collected on-farm data. However, proxy measures from routinely collected data are commonly used in animal breeding as indirect measures of selection for hard to

measure traits, and this can still enable us to model and quantify resistance and tolerance. This allows us to assess the benefits of selection, and to determine whether we should select for resistance, tolerance, or both.

Breeding for resistance and tolerance has been found to be sustainable, economically feasible and desirable, especially for common diseases that are unable to be controlled by vaccination and management practices (Stear et al., 2001). This is an animal welfare and industry-friendly approach that should be explored to meet our increasing need for positive changes in pork production methods, as it can improve the health and welfare of pigs, whilst maintaining productivity.

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REFERENCES

Abdi, H. (2010). Partial least squares regression and projection on latent structure regression (PLS regression). *Wiley Interdiscip. Rev. Comput. Stat.* 2, 97–106.

Adamo, S. A. (2004). How should behavioural ecologists interpret measurements of immunity? *Anim. Behav.* 68, 1443–1449.

Albers, G. A. A., Gray, G. D., Piper, L. R., Barker, J. S. F., Le Jambre, L. F., and Barger, I. A. (1987). The genetics of resistance and resilience to *Haemonchus contortus* infection in young merino sheep. *Int. J. Parasitol.* 17, 1355–1363.

Atkins, K. D., and Mortimer, S. I. (1989). *Project K/1/1065 Genetic Improvement of Reproductive Rate in Merino Sheep. Final Report to the Australian Wool Corporation.* Trangie: New South Wales Department of Agriculture, Agricultural Research Centre.

Baucom, R. S., and de Roode, J. C. (2011). Ecological immunology and tolerance in plants and animals. *Funct. Ecol.* 25, 18–28.

Bergstrom, K. S., Sham, H. P., Zarepour, M., and Vallance, B. A. (2012). Innate host responses to enteric bacterial pathogens: a balancing act between resistance and tolerance. *Cell. Microbiol.* 14, 475–484.

Best, A., White, A., and Boots, M. (2008). Maintenance of host variation in tolerance to pathogens and parasites. *Proc. Natl. Acad. Sci. U.S.A.* 105, 20786–20791.

Bishop, S. C. (2010). "Modelling farm animal diseases," in *Breeding for Disease Resistance in Farm Animals, 3rd Edn,* eds S. C. Bishop, R. F. E. Axford, F. W. Nicholas and J. B. Owen (Wallingford, Oxfordshire: CAB International), 38–54.

Bishop, S. C., Chesnais, J., and Stear, M. J., (2002). "Breeding for disease resistance: issues and opportunities," in *Seventh World Congress on Genetics Applied to Livestock Production* (Montpellier, France).

Bishop, S. C., and Mackenzie, K. A. (2003). Genetic management strategies for controlling infectious diseases in livestock populations. *Genet. Sel. Evol.* 35, S3–S17.

Bishop, S. C., and Woolliams, J. A. (2004). Genetic approaches and technologies for improving the sustainability of livestock production. *J. Sci. Food Agric.* 84, 911–919.

Bisset, S. A., and Morris, C. A. (1996). Feasibility and implications of breeding sheep for resilience to nematode challenge. *Int. J. Parasitol.* 26, 857–868.

Black, J. L., Giles, L. R., Wynn, P. C., Knowles, A. G., Kerr, C. A., Jones, M. R., et al. (2001). "A review-factors limiting the performance of growing pigs in commercial environments," in *Eighth Biennial Conference of the Australiasian Pig Science Association* (APSA), ed P. D. Cranwell (Adelaide, South Australia).

Blecha, F. (1998). "Immunological aspects: comparison with other species," in *The Lactating Sow*, eds P. J. Moughan, J. W. Schrama and M. W. A. Verstegen (Wageningen: Wageningen Pers), 23–44.

Boddicker, N., Waide, E. H., Rowland, R. R. R., Lunney, J. K., Garrick, D. J., Reecy, J. M., et al. (2012). Evidence for a major QTL associated with host response to porcine reproductive and respiratory syndrome virus challenge. *J. Anim. Sci.* 90, 1733–1746.

Buehler, D. M., Tieleman, B. I., and Piersma, T. (2010). How do migratory species stay healthy over the annual cycle? A conceptual model for immune function and for resistance to disease. *Integr. Comp. Biol.* 50, 346–357.

Cecchinato, A., Bonfatti, V., Gallo, L., and Carnier, P. (2008). Survival analysis of preweaning piglet survival in a dry-cured ham-producing crossbred line. *J. Anim. Sci.* 86, 2486–2495.

Clapperton, M., Diack, A. B., Matika, O., Glass, E. J., Gladney, C. D., Mellencamp, M. A., et al. (2009). Traits associated with innate and adaptive immunity in pigs: heritability and associations with performance under different health status conditions. *Genet. Sel. Evol.* 41, 54.

Clapperton, M., Glass, E. J., and Bishop, S. C. (2008). Pig peripheral blood mononuclear leucocyte subsets are heritable and genetically correlated with performance. *Animal* 2, 1575–1584.

Detilleux, J. C. (2011). Effectiveness analysis of resistance and tolerance to infection. *Genet. Sel. Evol.* 43, 9.

Doeschl-Wilson, A. B., and Kyriazakis, I. (2012). Should we aim for genetic improvement in host resistance or tolerance to infectious pathogens? *Front. Gene.* 3:272. doi: 10.3389/fgene.2012.00272

Doeschl-Wilson, A. B., Kyriazakis, I., Vincent, A., Rothschild, M. F., Thacker, E., and Galina-Pantoja, L. (2009). Clinical and pathological responses of pigs from two genetically diverse commercial lines to porcine reproductive and respiratory syndrome virus infection. *J. Anim. Sci.* 87, 1638–1647.

Doeschl-Wilson, A. B., Villanueva, B., and Kyriazakis, I. (2012). The first step towards genetic selection for host tolerance to infectious pathogens. *Front. Gene.* 3:265. doi: 10.3389/fgene.2012.00265

Flori, L., Gao, Y., Oswald, I. P., Lefevre, F., Bouffaud, M., Mercat, M.-J., et al. (2011). Deciphering the genetic control of innate and adaptive immune responses in pig: a combined genetic and genomic study. *BMC Proc.* 5(Suppl. 4):S32. doi: 10.1186/1753-6561-5-S4-S32

Gibbons, R. A., Sellwood, R., Burrows, M., and Hunter, P. A. (1977). Inheritance of resistance to neonatal, *E. coli* diarrhoea in the pig: examination of the genetic system. *Theor. Appl. Genet.* 51, 65–70.

Gray, G. D. (1997). The use of genetically resistance sheep to control nematode parasitism. *Vet. Parasitol.* 72, 345–366.

Henryon, M., Heegaard, P. M. H., Nielsen, J., Berg, P., and Juul-Madsen, H. R. (2006). Immunological traits have the potential to improve selection of pigs for resistance to clinical and subclinical disease. *Anim. Sci.* 82, 597–606.

Kaiser, P. (2010). "The immune system," in *Breeding for Disease Resistance in Farm Animals, 3rd Edn,* eds S. C. Bishop, R. F. E. Axford, F. W. Nicholas and J. B. Owen (Wallingford, Oxfordshire: CAB International), 15–37.

Kanis, E., De Greef, K. H., Hiemstra, A., and van Arendonk, J. A. M. (2005). Breeding for societally important traits in pigs. *J. Anim. Sci.* 83, 948–957.

Kause, A. (2011). Genetic analysis of tolerance to infections using random regressions: a simulation study. *Genet. Res.* 93, 291–302.

Kemper, K. E., Goddard, M. E., and Bishop, S. C. (2010). "A simple model of worm adaptation to livestock bred for improved worm resistance," in *Ninth World Congress on Genetics Applied to Livestock Production* (Leipzig, Germany).

Kerr, C. A., and Hines, B. M. (2005). The potential of new genetic technologies in selecting for stress resistance in pigs. *Aust. J. Exp. Agric.* 45, 775–782.

Knap, P. W. (2005). Breeding robust pigs. *Aust. J. Exp. Agric.* 45, 763–773.

Knap, P. W. (2012). "Pig breeding for increased sustainability," in *Encyclopedia of Sustainability Science and Technology*, ed R. A. Meyers (Germany: Springer), 7972–8012.

Knap, P. W., and Su, G. (2008). Genotype by environment interaction for litter size in pigs as quantified by reaction norms analysis. *Animal* 2, 1742–1747.

Leach, L. M., Ellis, M., Sutton, D. S., McKeith, F. K., and Wilson,

E. R. (1996). The growth performance, carcass characteristics, and meat quality of halothane carrier and negative pigs. *J. Anim. Sci.* 74, 934–943.

Lewis, C. R. G., Ait-Ali, T., Clapperton, M., Archibald, A. L., and Bishop, S. C. (2007). Genetic perspectives on host responses to Porcine Reproductive and Respiratory Syndrome (PRRS). *Viral Immunol.* 20, 343–357.

Lewis, C. R. G., Torremorell, M., Galina-Pantoja, L., and Bishop, S. C. (2009). Genetic parameters for performance trais in commercial sows estimated before and after an outbreak of porcine reproductive and respiratory syndrome. *J. Anim. Sci.* 87, 876–884.

Lunney, J. K., and Chen, H. (2010). Genetic control of host resistance to porcine reproductive and respiratory syndrome virus (PRRSV) infection. *Virus Res.* 154, 161–169.

Lynch, M., and Walsh, B. (1998). *Genetics and Analysis of Quantitative Traits.* Sunderland, MA: Sinauer.

Madec, F., Kobisch, M., and Leforban, Y. (1993). An attempt at measuring health in nucleus and multiplier pig farms. *Livest. Prod. Sci.* 34, 281–294.

Mallard, B. A., Wilkie, B. N., Kennedy, B. W., and Quinton, M. (1992). Use of estimated breeding values in a selection index to breed Yorkshire pigs for high and low immune and innate resistance factors. *Anim. Biotechnol.* 3, 257–280.

Medzhitov, R., Schneider, D. S., and Soares, M. P. (2012). Disease tolerance as a defense strategy. *Science* 335, 936–941.

Merks, J. W. M., Mathur, P. K., and Knol, E. F. (2012). New phenotypes for new breeding goals in pigs. *Animal* 6, 535–543.

Mérour, I., Hermesch, S., Schwob, S., and Tribout, T. (2009). "Effect of the halothane genotype on growth performances, carcase and meat quality traits in the Pietrain Breed of the French National Pig Breeding Program," in *Eighteenth Conference of the Association for the Advancement of Animal Breeding and Genetics* (Barossa Valley, South Australia).

Miller, M. R., White, A., and Boots, M. (2005). The evolution of host resistance: tolerance and control as distinct strategies. *J. Theor. Biol.* 236, 198–207.

Miller, M. R., White, A., and Boots, M. (2006). The evolution of parasites in response to tolerance in their hosts: the good, the bad, and apparent commensalism. *Evolution* 60, 945–956.

Morris, C. A., Amyes, N. C., Bisset, S. A., and Mackay, A. D. (2004). Resilience to nematode parasite challenge in industry and AgResearch selection flocks. *Proc. N.Z. Soc. Anim. Prod.* 64, 300–303.

Morris, C. A., Bisset, S. A., Vlassoff, A., Wheeler, M., West, C. J., Devantier, B. P., et al. (2010). Selecting for resilience in Romney sheep under nematode parasite challenge, 1994–2007. *N.Z. J. Agric. Res.* 53, 245–261.

Pilson, D. (2000). The evolution of plant response to herbivory: simultaneously considering resistance and tolerance in *Brassica rapa*. *Evol. Ecol.* 14, 457–489.

Potter, M. L., Tokach, L. M., and Dritz, S. S. (2012). Genetic line influences pig growth rate responses to vaccination for porcine circovirus type 2. *J. Swine Health Prod.* 20, 34–43.

Prunier, A., Heinonen, M., and Quesnel, H. (2010). High physiological demands in intensively raised pigs: impact on health and welfare. *Animal* 4, 886–898.

Råberg, L., Graham, A. L., and Read, A. F. (2009). Decomposing health: tolerance and resistance to parasites in animals. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* 364, 37–49.

Råberg, L., Sim, D., and Read, A. F. (2007). Disentangling genetic variation for resistance and tolerance to infectious diseases in animals. *Science* 318, 812–814.

Rauw, W. M., Kanis, E., Noordhuizen-Stassen, E. N., and Grommers, F. J. (1998). Undesirable side effects of selection for high production efficiency in farm animals: a review. *Livest. Prod. Sci.* 56, 15–33.

Read, A. F., Graham, A. L., and Råberg, L. (2008). Animal defenses against infectious agents: is damage control more important than pathogen control? *PLoS Biol.* 6:e1000004. doi: 10.1371/journal.pbio.1000004

Reed, S. N., and McGlone, J. J. (2000). Immune status of PIC Camborough-15 sows, 25% Meishan sows, and their offspring kept indoors and outdoors. *J. Anim. Sci.* 78, 2561–2567.

Roff, D. A. (1997). *Evolutionary Quantitative Genetics*. New York, NY: Chapman and Hall.

Rohr, J. R., Raffel, T. R., and Hall, C. A. (2010). Developmental variation in resistance and tolerance in a multi-host-parasite system. *Funct. Ecol.* 24, 1110–1121.

Rothschild, M. F. (1998). "Selection for disease resistance in the pig," in *National Swine Improvement Federation Conference* (East Lansing Marriot, East Lansing, MI).

Roy, B. A., and Kirchner, J. W. (2000). Evolutionary dynamics of pathogen resistance and tolerance. *Evolution* 54, 51–63.

Ruppert, D., Carroll, R. J., and Wand, M. P. (2003). *Semiparametric Regression.* Cambridge, NY: Cambridge University Press.

Salak-Johnson, J. L., and McGlone, J. J. (2007). Making sense of apparently conflicting data: stress and immunity in swine and cattle. *J. Anim. Sci.* 85, E81–E88.

Sather, A. P., Murray, A. C., Zawadski, S. M., and Johnson, P. (1991). The effect of the halothane gene on pork production and meat quality of pigs reared under commercial conditions. *Can. J. Anim. Sci.* 71, 959–967.

Schinckel, A. P., Richert, B. P., Frank, J. W., and Kendall, D. C. (1999). "Genetic by environmental interactions for pig growth," in *Purdue University Swine Day Report* (Purdue: Purdue University).

Schneider, D. S., and Ayres, J. S. (2008). Two ways to survive infection: what resistance and tolerance can teach us about treating infectious diseases. *Nat. Rev. Immunol.* 8, 889–895.

Simms, E. L., and Triplett, J. (1994). Costs and benefits of plant responses to disease: resistance and tolerance. *Evolution* 48, 1973–1985.

Stear, M. J., Bishop, S. C., Mallard, B. A., and Raadsma, H. (2001). The sustainability, feasibility and desirability of breeding livestock for disease resistance. *Res. Vet. Sci.* 71, 1–7.

Svensson, E. I., and Råberg, L. (2010). Resistance and tolerance in animal enemy-victim coevolution. *Trends Ecol. Evol.* 25, 267–274.

Wilkie, B., and Mallard, B. (1999). Selection for high immune response: an alternative approach to animal health maintenance? *Vet. Immunol. Immunopathol.* 72, 231–235.

Williams, N. H. (1998). "Impact of immune system activation on pig growth and animo acid needs," in *Progress in Pig Science,* eds J. Wiseman, M. A. Varley, and J. P. Chadwick (Nottingham, UK: Nottingham University Press), 583–592.

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Control of porcine reproductive and respiratory syndrome (PRRS) through genetic improvements in disease resistance and tolerance

Raymond R. R. Rowland[1]*, Joan Lunney[2] and Jack Dekkers[3]

[1] *Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS, USA*
[2] *Animal Parasitic Diseases Laboratory, United State Department of Agriculture, Agricultural Research Services, Beltsville Agricultural Research Center, Beltsville, MD, USA*
[3] *Department of Animal Science, Iowa State University, Ames, IA, USA*

Edited by:
Andrea B Doeschl-Wilson, The University of Edinburgh, UK

Reviewed by:
Steve Bishop, The University of Edinburgh, UK
Antti Kause, MTT Agrifood Research Finland, Finland
Gerald Reiner, Justus-Liebig-University, Germany

****Correspondence:***
Raymond R. R. Rowland, Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, 1800 Denison Ave., Kansas State University, Manhattan, KS 66506, USA.
e-mail: browland@vet.k-state.edu

Infections caused by porcine reproductive and respiratory syndrome virus (PRRSV) have a severe economic impact on pig production in North America, Europe, and Asia. The emergence and eventual predominance of PRRS in the 1990s are the likely result of changes in the pork industry initiated in the late 1970s, which allowed the virus to occupy a unique niche within a modern commercial production system. PRRSV infection is responsible for severe clinical disease, but can maintain a life-long subclinical infection, as well as participate in several polymicrobial syndromes. Current vaccines lessen clinical signs, but are of limited use for disease control and elimination. The relatively poor protective immunity following vaccination is a function of the virus's capacity to generate a large degree of genetic diversity, combined with several strategies to evade innate and adaptive immune responses. In 2007, the PRRS Host Genetics consortium (PHGC) was established to explore the role of host genetics as an avenue for PRRS control. The PHGC model for PRRS incorporates the experimental infection of large numbers of growing pigs and has created the opportunity to study experimental PRRSV infection at the population level. The results show that pigs can be placed into distinct phenotypic groups, including pigs that show resistance (i.e., low virus load) or pigs that exhibit "tolerance" to infection. Tolerance was illustrated by pigs that gain weight normally in the face of a relatively high virus load. Genome-wide association analysis has identified a region on chromosome 4 (SSC4) correlated with resistance; i.e., lower cumulative virus load within the first 42 days of infection. The genomic region is near a family of genes involved in innate immunity. The region is also associated with higher weight gain in challenged pigs, suggesting that pigs with the resistance alleles don't seem to simultaneously experience reduction in growth, i.e., that resistance and tolerance are not antagonistically related. These results create the opportunity to develop breeding programs that will produce pigs with increased resistance to PRRS and simultaneously high growth rate. The identification of genomic markers involved in actual tolerance will likely prove more difficult, primarily because tolerance is difficult to quantify and because tolerance mechanism are still poorly understood. Another avenue of study includes the identification of genomic markers related to improved response following vaccination.

Keywords: porcine reproductive and respiratory syndrome, PRRS resistance, genome-wide association study

INTRODUCTION

Porcine reproductive and respiratory syndrome (PRRS) is the most economically significant disease impacting commercial pig production in North America, Europe, and Asia. PRRS was first described in 1987, followed by characterization of the PRRS virus (PRRSV) in Europe in 1991, and soon after in the US (Benfield et al., 1992). Clinical outcomes following infection include reproductive failure, respiratory disease in young pigs, and reduced growth performance (Keffaber, 1989; Zimmerman et al., 2006; Lunney et al., 2010). Perhaps the greatest impact is the maintenance of a relatively long-term subclinical infection which participates in a variety of polymicrobial syndromes, such as porcine respiratory disease complex (PRDC) and porcine circovirus associated disease (PCVAD). In the field, PRRSV continues to be linked to a variety of new disease syndromes. In 2005, the emergence of porcine high fever disease (PHFD) in China was linked to a novel PRRSV strain (Tong et al., 2007). Affected herds experienced high morbidity and in some cases 100% mortality. PHFD PRRSV continues to spread throughout Southeast Asia and has been linked to the co-infection of pigs with the *Reston ebolavirus* (Barrette et al., 2009; Rowland et al., 2012). A new PRRS virus, called Lena, is associated

with outbreaks of severe PRRS in Europe (Karniychuk et al., 2010).

PRRSV is a member of the arterivirus group, which includes lactate dehydrogenase–elevating virus (LDV) of mice, simian hemorrhagic fever virus (SHFV), and equine arteritis virus (EAV). The arteriviruses belong to the family, *Arteriviridae*, within the order, *Nidoviridales*. As a group, the arteriviruses possess several novel properties related to viral pathogenesis, including cytopathic replication in macrophages, the capacity to establish a persistent infection, and cause severe disease (Snijder and Spaan, 2007). The 15.4 kb PRRSV genome codes for at least 10 open reading frames (ORFs). The virion includes a nucleocapsid composed of a single nucleocapsid (N) protein. The viral envelope is dominated by the major glycoprotein, GP5, and the matrix (M) protein. Minor outer proteins include GP2, GP3, and GP4, along with two small proteins, E and ORF5 (Johnson et al., 2011). PRRSV is divided into European and North American genotypes, designated as type 1 and type 2, respectively. Even though type 1 and type 2 viruses were recognized almost simultaneously on the continents of North America and Europe and produce similar clinical signs, the two genotypes possess only about 70% identity at the nucleotide level. Nucleotide sequence diversity within each genotypic group can be as much as 10%. During the mid-1990s, viruses of type 2 origin were introduced into Europe. In 1999, type 1 viruses first appeared in North America (Fang et al., 2007).

The various clinical outcomes following PRRSV infection are a consequence of a complex set of interactions between the virus and the pig host. Following infection, viremia in young pigs continues for ∼28 days. During this time, the virus primarily targets macrophages in the lung. The inflammatory response resulting from the infection and removal of alveolar macrophages is responsible for the onset of acute respiratory signs. Following the disappearance of virus from the blood, virus replication continues within monocyte/macrophage cells in the lymphoid tissues, including tonsils and lymph nodes (Rowland et al., 2003). Virus can be isolated from lymph nodes for more than 100 days after infection and persistently infect pigs is easily transmit virus to naïve pigs, likely via shedding from tonsils. Virus replication in the host gradually decays until the virus becomes extinct, at around 200 days after infection (Horter et al., 2001; Rowland et al., 2003). The mechanism for virus extinction is not clear, but likely relates to the gradual disappearance of PRRSV-permissive cells combined with a partially effective immune response; e.g., low levels of circulating neutralizing antibody. By definition, PRRSV is not a "persistent" virus, but since the typical lifespan of a commercial production pig is approximately 180 days, PRRSV infection is considered to be "life-long."

The mechanistic basis for maintaining a life-long infection is dependent on a variety of factors, including; (1) a complex virion structure dominated by heavily glycosylated surface proteins, (2) the re-direction of the humoral response toward non-surface proteins, such as N and a variety of non-structural proteins, (3) antigenic and genetic drift within structural and non-structural genes, and (4) subversion of innate responses (Chand et al., 2012). Modified-live virus (MLV) and inactivated virus are the two principal approaches for PRRS vaccination. At least 20 PRRS vaccines are commercially available, worldwide. In general, inactivated virus vaccines are not effective. MLV vaccines are effective in protecting the pig from challenge with a genetically similar or "homologous" virus, but provide little protection against heterologous (genetically diverse) PRRSV isolates (Huang and Meng, 2010; Murtaugh and Genzow, 2011). The purpose of this review is to provide an overview of experimental models of PRRS infection, including the phenotypic properties of resistant and tolerant pigs. For the purpose of this review, PRRS "resistance" is defined as the ability of a host to limit pathogen burden, e.g., by inhibiting pathogen entry or restricting reproduction of the pathogen within the host and includes all mechanisms that limit the host pathogen burden. "Tolerance" is defined as the ability of a host to limit the detrimental impact of a given pathogen burden on the host's performance without directly affecting pathogen burden. Furthermore, for the purpose of this review, the definition includes the ability to maintain homeostasis in the presence of a replicating pathogen, with limited ensuing pathology.

THE ROLE OF THE HOST GENOME IN RESPONSE TO PRRSV INFECTION

Since the discovery of the PRRS virus, there has emerged a body of evidence associating host genetics with different outcomes following PRRSV infection. In 1998, Halbur et al. evaluated PRRSV infection in a variety of pig breeds and reported more PRRS-associated lung lesions in Hampshire pigs. On the reproductive side, Lowe et al. (2005) concluded that genetics influenced abortion rates in PRRSV-infected Sows. Using an *in vitro* approach, Vincent et al. (2006) reported that macrophage responses were partially predictive of breeds with increased PRRSV resistance. Petry et al. (2005) found that, compared to a Hampshire/Duroc line, a Large White/Landrace line showed reduced viremia when infected with PRRSV. In later work, the same group (Petry et al., 2007) found that pigs with lower viremia possessed higher levels of serum interleukin-8 prior to infection. Previous estimates of heritability of PRRSV resistance are scarce, but heritability estimates for the effect of PRRSV infection on the percentage of live pigs born to infected sows range from 0.12 to 0.15 (Lewis et al., 2009). A recent review by Lunney and Chen (2010) describes the latest progress on the genetics of disease resistance, including the application of new tools such as the 60K SNP chip for performing genome-wide association studies (GWASs).

Studies related to understanding the genetic basis for PRRSV tolerance are non-existent. However, previous studies have shown that the detrimental impact of PRRSV infection on growth varies between and within lines and breeds (Greiner et al., 2000; Petry et al., 2005; Doeschl-Wilson et al., 2009), which may be considered as indication that genetic variation in tolerance exists.

A good example of tolerance is found in another arterivirus, LDV. Within 24 h after infection of a mouse, LDV infection levels in the blood approach 10^{10} virus particles per ml; however, there are no clinical signs of infection. Viremia decreases to about 10^{7} virus particles and remains at that level for the remainder of the mouse's life (Plagemann et al., 1995). The only evidence of infection is increased circulating lactate dehydrogenase (LDH), the result of a targeted elimination of a subpopulation of LDH-scavenging macrophages by LDV. The virus does not

target the macrophage precursor; therefore, the level of LDV is maintained at a steady state depending on the production of new LDV-permissive macrophages. Normally, macrophages would be protected by the presence of virus-specific neutralizing antibody. Similar to PRRSV, the LDV-specific neutralizing antibody response is relatively weak, a consequence of a complex virion structure, including large quantities of surface protein glycosylation. Since the mouse does not become immunocompetent until after birth, mice can be made immunologically tolerant to LDV, by infecting neonates within 24 h after birth. The outcome of neonatal infection is the absence of a LDV-specific antibody response, a demonstration of immunological tolerance. However, in mice made immunologically tolerant to LDV, there is no alteration in the level of virus in the blood and no change in the course of viremia. Furthermore, the immunologically tolerant mice do not exhibit clinical disease signs (Rowland et al., 1994). Therefore, mice are "tolerant" to LDV infection. Tolerance to LDV infection is a mechanism that has the least impact on evolutionary fitness of the host. In a similar manner, a PRRSV tolerant pig would likely possess a relatively high virus load, but would show no pathology or clinical signs related to disease, including little reduction in growth or reproductive traits. In the real world, a PRRS tolerant pig would be particularly beneficial in high-density pig growing regions where PRRS is endemic and difficult to control. However, one unintended consequence of a pig showing tolerance would be the continuous shedding of virus, an efficient mechanism for spreading virus to naïve pigs.

In 2007, it was generally recognized by commodity groups, industry, and scientific communities that the next generation of improved PRRS vaccines was still years away and that genetic improvement offered a logical solution. In response, the National Pork Board supported the formation of the PRRS Host Genetics Consortium (PHGC). The PHGC was formed as a mechanism for conducting the scientific research necessary to elucidate the role of the host genome in the response of pigs to PRRSV infection. The ultimate goal is to find genomic markers that can be employed in the development of breeding programs to lessen the impact of PRRSV on the commercial pig industry. Genetic improvement does not offer a single "magic bullet" solution, but would be an integral component of other disease management strategies. For example, identification of genomic markers associated with enhanced protection after vaccination could be used to select for, and, so-called "vaccine-ready" pigs. As discussed above, pigs tolerant to PRRSV infection would offer a solution for regions with high pig densities where disease control is difficult. And finally, markers associated with susceptibility to disease would be useful to avoid the unintended consequences that can occur when breeding pigs for other desirable traits.

EXPERIMENTAL MODELS FOR INVESTIGATING THE ROLE OF THE HOST GENOME IN RESPONSES TO PRRSV INFECTION

For livestock species, investigating the association between genomic markers and the host response to infection typically incorporates hundreds, if not thousands, of infected animals. In the field, these numbers are readily achieved on affected farms by collecting phenotypic data, such as virus infection status (infected versus not infected animals), morbidity/mortality, and the presence or absence of clinical signs. Even though field data are highly relevant, assessing phenotypic traits associated with PRRSV infection can be complicated by several factors. For instance, obtaining only a single measure of infection status cannot be used to establish when the pig was first exposed or whether the infection is acute or chronic. Furthermore, the presence of other pathogens circulating within the population can mimic or mask PRRS clinical signs. For example, infection by influenza virus can mimic PRRSV respiratory clinical signs. Other complicating factors include the unknown properties of a particular PRRSV field isolate, the contribution of the environmental factors and overall health status.

The use of experimental infection models can eliminate or minimize the shortcomings of field studies. For instance, performing repeated phenotypic measurements following experimental challenge with a defined virus can yield reproducible and accurate determinations of virus-related traits, such as virus load, peak viremia, and viral clearance from the blood. Disease-related impacts on growth and performance can also be accurately measured. Other factors, such as nutrition and environment can be easily controlled. However, there are important considerations when performing experimental studies. For example, achieving the desired number of animals can be expensive. Another consideration is that experimentally infected animals maintained under "pristine" environmental conditions do not reproduce the environment found on the typical farm. Therefore, a particular experimental model may not reflect the response of animals maintained in the field.

Models that reproduce the effect of PRRSV on pregnant sows have been described in the literature (Rowland et al., 2003; Rowland, 2010). Measurable outcomes include the number of abortions or dead pigs. However, conducting pregnant sow studies on a large scale can be complex and prohibitively expensive. Another experimental PRRS model, often described in the literature measures the impact of PRRSV infection on the severity of respiratory disease in young pigs. Phenotypic disease traits include measurements of lung lesions scores and the amount of virus in alveolar macrophages, obtained by lung lavage. Pigs are experimentally infected and lungs removed between 7 and 15 days after infection. Phenotypic disease traits, such as lung lesions scores are obtained post-mortem. One limitation to this approach is the subjective nature for assessing lung pathology, which requires a pathologist or other trained professional to make the lung lesion determinations. Furthermore, different observers can obtain different disease scores for the same animal. The terminal nature of the experimental model prevents the collection of repeated measurements of lung lesion development and the resolution in the same pig.

The model developed by the PHGC incorporates a nursery pig model, described in Boddicker et al. (2012). High-health pigs are obtained from crossbred commercial lines with complete parentage and pedigree records. Pigs are negative for PRRSV, *Mycoplasma hyopneumoniae*, swine influenza virus (SIV), and porcine circovirus type 2 (PCV2). Each challenge trial group is comprised of a population of 200 pigs from at least 30 litters (six pigs per litter), which are derived from a minimum of 10 sires mated with 3–8 dams/sire. There is no pre-selection of

sires or dams for any PRRS-related trait. Piglets and parents are genotyped for >60,000 single-nucleotide polymorphisms (SNPs) using the Porcine SNP60 BeadChip (Illumina). All phenotypic and genotypic data are stored and made available to the PHGC membership through a secure relational database (http://www.animalgenome.org/lunney/index.php).

Pigs, at 3–4 weeks of age, are challenged with a well-characterized PRRSV isolate. Infection and disease-related phenotypic traits are collected for 42 days after infection. The 42 day period covers both the acute and early persistent stages of PRRSV infection. For the purpose of definition, virus recovered from tonsil or other lymphoid tissues at 42 days of infection is the result of "persistence." Virus load and weight gain are the two principal quantitative phenotypic traits measured, each reflecting important aspects of PRRSV infection. Virus load relates to amount of virus replication and reflects the potential for a pig to spread virus. Virus load is measured as the area under the curve of viremia measurements taken over the first 21 days after infection. Weight gain is used as measurement of the impact of that PRRSV infection has on growth performance. Both traits are quantifiable, easily reproducible, and do not require a high level of expertise to measure. Additional phenotypic data include measurements of innate and adaptive immunity, mortality, and the amount of virus in tonsil at 42 days.

PHENOTYPIC RESPONSES TO PRRSV INFECTION

The course of viremia and weight gain after experimental PRRSV infection are described by Boddicker et al. (2012). **Figure 1** shows an example of PRRSV RT-PCR results for 166 pigs in a single experimental infection trial. In this example, viremia peaked between 7 and 14 days after infection and then declined. As expected, by 28 days post-infection, serum virus declined to undetectable levels in most pigs. However, in a subpopulation consisting of ∼10–20% of pigs, circulating virus reappeared. Virus rebound following PRRSV infection is a phenomenon previously reported by Reiner et al. (2010). The mechanism for virus rebound is unclear, but could represent the emergence of immune escape variants.

Pigs in the PHGC model showed a wide variation in weight gain, with some pigs gaining weight at a relatively normal rate, while others failed to thrive during the 42 day infection period (**Figure 2**). One hypothesis is that pigs that gain weight normally are the best at controlling virus infection. To address this possibility, a plot showing weight gain versus virus load is presented in **Figure 3**. The pigs falling at each of the four

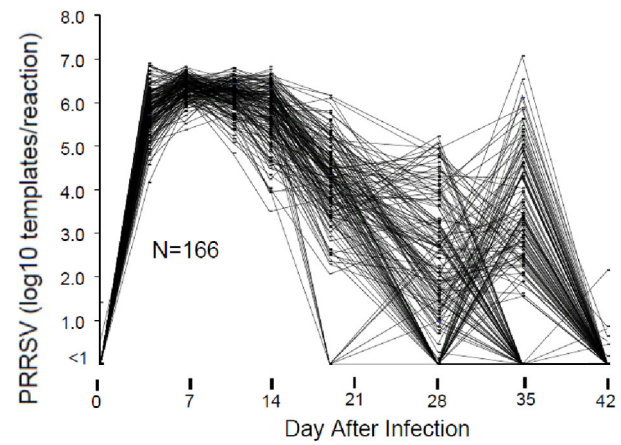

FIGURE 1 | Viremia following PRRSV infection. Viremia was measured by RT-PCR of viral RNA using a commercial diagnostic PRRSV assay. For the purpose of standardization, the results were reported as number of PRRSV templates per 50 ul PCR reaction. Results are shown for those pigs in a single 200 pig trial that possessed data for all days.

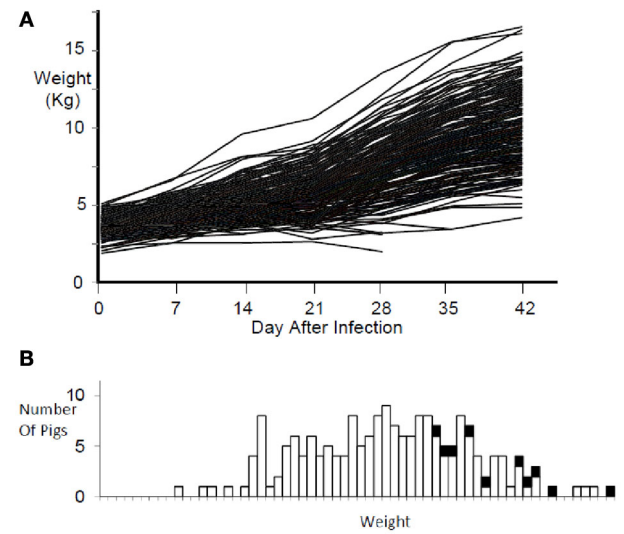

FIGURE 2 | Weight gain during PRRSV infection. Panel **(A)** shows the weight gain for individual infected pigs for 42 days. Panel **(B)** shows the weight distribution at 42 days after infection for the same pigs in panel **(A)**. Black squares represent non-infected reference pigs.

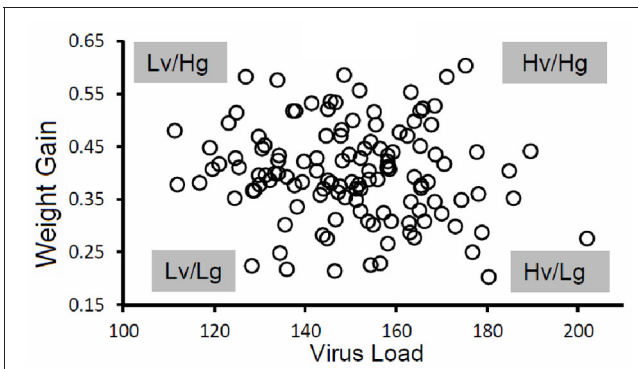

FIGURE 3 | Virus load versus weight gain. The figure shows results for a single trial. The virus load was calculated as the area under the curve for viremia over the first 21 days for each pig as described in **Figure 1**. Average daily gain was calculated as the weight at 42 days after infection minus the weight on the day of virus challenge divided by 42 days. Key: Hv, high virus load; Lv, low virus load; Hg, high weight gain; Lg, low weight gain. Virus load for each pig was determined by calculating the area under the curve for the first 21 days after infection.

extremes of the scatter plot can be described as: high virus/low weight gain (Hv/Lg), high virus/high weight gain (Hv/Hg), Low virus/Low weight gain (Lv/Lg), Low virus/High gain (Lv/Hg). Approximately 10% of pigs fall into each of the extreme groups. Pigs in the Lv/Hg group can be described as "resistant" to the effects of PRRSV; whereas, Hv/Lg pigs are sensitive to infection. Pigs in the Hv/Hg qroup provide the best evidence for a subgroup of pigs that may be considered as "PRRSV tolerant," i.e., retain normal growth in the presence of a relatively high virus load. However, caution is advised in the interpretation of the results as high growth rates alone are not necessarily reliable indicators of tolerance. In order to obtain unbiased tolerance estimates growth rate measures of the same pigs both infected and in the absence of the infection would need to be integrated into the appropriate statistical framework (e.g., Kause, 2011; Doeschl-Wilson et al., 2012).

GENOMIC MARKERS RELATED TO WEIGHT GAIN AND VIRUS LOAD

To date, 11 groups of 200 pigs from seven genetic sources have been evaluated under the PHGC model. Results for the genetic analysis of the first 3 groups (PHGC1-3) from a single genetic source, are reported in Boddicker et al. (2012). The estimated heritabilities are 0.3 for both viral load and weight gain after challenge with the PRRSV isolate NVSL 97-7985 (Boddicker et al., 2012). A GWAS incorporating the 60 K SNP chip identified genomic regions associated with viral load on chromosomes 4 (SSC4) and SSCX regions on chromosomes SSC1, SSC4, SSC7, and SSC17 were associated with weight gain. Furthermore, both virus load and weight gain were associated with a single genomic region in SSC4, which is best represented by a single SNP marker, WUR10000125 (WUR). The 1 Mb region in SSC4, which exhibits strong linkage disequilibrium, explained 15.7% of the genetic variance for viral load and 11.2% for weight gain. The estimated effects for this region were favorably and nearly perfectly correlated; i.e., pigs with low virus load exhibited greater weight gain. The favorable allele (B) had a frequency of 0.16 within the experimental population of pigs. Although the number of individuals with the BB genotype was present at a low frequency, the B allele appeared to be dominant, i.e., pigs with the AB genotype showed a favorable response compared to AA.

Candidate genes near the WUR SNP include the guanylate-binding protein (GBP) gene family [reviewed in Vestal and Jeyaratnam (2011)]. GBPs are induced by cytokines, such as interferon, and are unique in their ability to bind guanylate. In mice, the family consists of 11 genes. Expression of GBP is associated with defense against a variety of RNA viruses, including hepatitis C virus, vesicular stomatitis virus, and encephalomyocarditis virus. The mechanism of how GBP might inhibit PRRSV replication or influence growth are unclear. The marker on SSCX, which was associated with only virus load, is in the region of CHST7, another gene with antiviral properties (Nyberg et al., 2004).

The results reported by Boddicker et al. (2012) provide the first clear evidence for a genomic marker linked to the response of the host to PRRSV infection and create the possibility to breed pigs for increased resistance to infection and improved performance. Unexpectedly, both disease traits converged at a single marker. In the experimental studies, genotype BB and AB pigs exhibited as much as 10% greater weight gain compared to the predominant AA genotype. This benefit is highly significant in an industry that survives on small profit margins. Future work is directed at determining if similar differences between AA and BB animals occur under field conditions as well as the investigation of other markers associated with disease resistance or tolerance.

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REFERENCES

Barrette, R. W., Metwally, S. A., Rowland, J. M., Xu, L., Zaki, S. R., Nichol, S. T., et al. (2009). Discovery of swine as a host for the *Reston ebolavirus*. *Science* 325, 204–206.

Benfield, D. A., Nelson, E., Collins, J. E., Harris, L., Goyal, S. M., Robison, D., et al. (1992). Characterization of swine infertility and respiratory syndrome (SIRS) virus (isolate ATCC VR-2332). *J. Vet. Diagn. Invest.* 4, 127–133.

Boddicker, N., Waide, E. H., Rowland, R. R. R., Lunney, J. K., Garrick, D. J., Reecy, J. M., et al. (2012). Evidence for a major QTL associated with host response to Porcine Reproductive and Respiratory Syndrome virus challenge. *J. Anim. Sci.* 90, 1733–1746.

Chand, R., Trible, B., and Rowland, R. (2012). Pathogenesis of porcine reproductive and respiratory syndrome virus. *Curr. Opin. Virol.* 2, 256–263.

Doeschl-Wilson, A. B., Kyriazakis, I., Vincent, A., Rothschild, M. F., Thacker, E., and Galina-Pantoja, L. (2009). Clinical and pathological responses of pigs from two genetically diverse commercial lines to porcine reproductive and respiratory syndrome virus infection. *J. Anim. Sci.* 87, 1638–1647.

Doeschl-Wilson, A. B., Villanueva, B., and Kyriazakis, I. (2012). The first step towards genetic selection for host tolerance to infectious pathogens: obtaining the tolerance phenotype through group estimates. *Front. Gene.* 3:265. doi: 10.3389/fgene.2012.00265

Fang, Y., Schneider, P., Zhang, W. P., Faaberg, K. S., Nelson, E. A., and Rowland, R. R. (2007). Diversity and evolution of a newly emerged North American Type 1 porcine arterivirus: analysis of isolates collected between 1999 and 2004. *Arch. Virol.* 152, 1009–1017.

Greiner, L. L., Stahly, T. S., and Stabel, T. J. (2000). Quantitative relationship of systemic virus concentration on growth and immune response in pigs. *J. Anim. Sci.* 78, 2690–2695.

Horter, D. C., Pogranichniy, R. M., Chang, C. C., Evans, R. B., Yoon, K. J., and Zimmerman, J. J. (2001). Characterization of the carrier state in porcine reproductive and respiratory syndrome virus infection. *Vet. Microbiol.* 86, 213–228.

Huang, Y. W., and Meng, X. J. (2010). Novel strategies and approaches to develop the next generation of vaccines against porcine reproductive and respiratory syndrome virus (PRRSV). *Virus Res.* 154, 141–149.

Johnson, C. R., Griggs, T. F., Gnanandarajah, J., and Murtaugh, M. P. (2011). Novel structural protein in porcine reproductive and respiratory syndrome virus encoded by an alternative ORF5 present in all arteriviruses. *J. Gen. Virol.* 92, 1107–1116.

Karniychuk, U. U., Geldhof, M., Vanhee, M., Van Doorsselaere, J., Saveleva, T. A., and Nauwynck, H. J. (2010). Pathogenesis and antigenic characterization of a new East European subtype 3 porcine reproductive and respiratory syndrome virus isolate. *BMC Vet. Res.* 6:30. doi: 10.1186/1746-6148-6-30

Kause, A. (2011). Genetic analysis of tolerance to infections using random regressions: a simulation study. *Genet. Res.* 93, 291–302.

Keffaber, K. K. (1989). Reproductive failure of unknown etiology. *Am.*

Assoc. Swine Prac. Newslett. 1, 1–10.

Lewis, C. R., Torremorell, M., Galina-Pantoja, L., and Bishop, S. C. (2009). Genetic parameters for performance traits in commercial sows estimated before and after an outbreak of porcine reproductive and respiratory syndrome. *J. Anim. Sci.* 87, 876–884.

Lowe, J. E., Husmann, R., Firkins, L. D., Zuckermann, F. A., and Goldberg, T. L. (2005). Correlation of cell-mediated immunity against porcine reproductive and respiratory syndrome virus with protection against reproductive failure in sows during outbreaks of porcine reproductive and respiratory syndrome in commercial herds. *J. Am. Vet. Med. Assoc.* 226, 1707–1711.

Lunney, J. K., Benfield, D. A., and Rowland, R. R. (2010). Porcine reproductive and respiratory syndrome virus: an update on an emerging and re-emerging viral disease of swine. *Virus Res.* 154, 1–6.

Lunney, J. K., and Chen, H. (2010). Genetic control of host resistance to porcine reproductive and respiratory syndrome virus (PRRSV) infection. *Virus Res.* 154, 161–169.

Murtaugh, M. P., and Genzow, M. (2011). Immunological solutions for treatment and prevention of porcine reproductive and respiratory syndrome (PRRS). *Vaccine* 29, 8192–8204.

Nyberg, K., Ekblad, M., Bergstrom, T., Freeman, C., Parish, C. R., Ferro, V., et al. (2004). The low molecular weight heparin sulfate-mimetic, PI-88, inhibits cell-to-cell spread of herpes simplex virus. *Antiviral Res.* 63, 15–24.

Petry, D. B., Holl, J. W., Weber, J. S., Doster, A. R., Osorio, F. A., and Johnson, R. K. (2005). Biological responses to porcine respiratory and reproductive syndrome virus in pigs of two genetic populations. *J. Anim. Sci.* 83, 1494–1502.

Petry, D. B., Lunney, J., Boyd, P., Kuhar, D., Blankenship, E., and Johnson, R. K. (2007). Differential immunity in pigs with high and low responses to porcine reproductive and respiratory syndrome virus infection. *J. Anim. Sci.* 85, 2075–2092.

Plagemann, P. G. W., Rowland, R. R. R., Even, C., and Faaberg, K. S. (1995). Lactate dehydrogenase-elevating virus: an ideal persistent virus? *Springer Semin. Immunopathol.* 17, 167–186.

Reiner, G., Pesch, S., and Ohlinger, V. F. (2010). Variation in resistance to the porcine reproductive and respiratory syndrome virus in pietrain and miniature pigs. *J. Anim. Breed. Genet.* 127, 100–106.

Rowland, J., Geisbert, T., and Rowland, R. R. R. (2012). "Ebolavirus," in *Diseases of Swine*, eds J. Zimmerman, A. Ramirez, G. Stevenson, L. Karricker and K. Schwartz (West Sussex, UK: Wiley-Blackwell), 525–527.

Rowland, R. R. (2010). The interaction between PRRSV and the late gestation pig fetus. *Virus Res.* 154, 114–122.

Rowland, R. R. R., Even, C., Anderson, G. W., Chen, Z., Hu, B., and Plagemann, P. G. W. (1994). Neonatal infection of mice with lactate dehydrogenase-elevating virus (LDV) results in suppression of humoral antiviral immune response but does not alter the course of viremia, polyclonal activation of B cells and immune complex formation. *J. Gen. Virol.* 75, 1071–1079.

Rowland, R. R. R., Lawson, S., Rossow, K., and Benfield, D. A. (2003). Lymphoid tissue tropism of porcine reproductive and respiratory syndrome virus replication during persistent infection of pigs originally exposed to virus *in utero*. *Vet. Microbiol.* 96, 219–235.

Snijder, E. J., and Spaan, W. J. M. (2007). "Arteriviruses," in *Fields Virology*, eds M. Knipe and M. Howley (Philadelphia, PA: Lippincott Williams and Wilkins), 1337–1355.

Tong, G. Z., Zhou, Y. J., Hao, X. F., Tian, Z. J., An, T. Q., and Qiu, H. J. (2007). Highly pathogenic porcine reproductive and respiratory syndrome, China. *Emerg. Infect. Dis.* 13, 1434–1436.

Vestal, D. J., and Jeyaratnam, J. A. (2011). The guanylate-binding proteins: emerging insights into the biochemical properties and functions of this family of large interferon-induced guanosine triphosphatase. *J. Interferon Cytokine Res.* 31, 89–97.

Vincent, A. L., Thacker, B. J., Halbur, P. G., Rothschild, M. F., and Thacker, E. L. (2006). An investigation of susceptibility to porcine reproductive and respiratory syndrome virus between two genetically diverse commercial lines of pigs. *J. Anim. Sci.* 84, 49–57.

Zimmerman, J., Benfield, D. A., Murtaugh, M. P., Osorio, F., Stevenson, G. W., and Torremorell, M. (2006). "Porcine reproductive and respiratory syndrome virus (porcine arterivirus)," in *Diseases of Swine,* eds B. Straw, D. Taylor, J. Zimmeran and S. D'Allaire (West Sussex, UK: Wiley-Blackwell), 387–418.

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Toward integrative genomics study of genetic resistance to *Salmonella* and *Campylobacter* intestinal colonization in fowl

Fanny Calenge[1]* and Catherine Beaumont[2]

[1] INRA, UMR1313 Génétique Animale et Biologie Intégrative, Jouy-en-Josas cedex, France
[2] INRA, UR083 Unité de Recherches Avicoles, Nouzilly, France

Edited by:
Andrea B. Doeschl-Wilson, The University of Edinburgh, UK

Reviewed by:
Eileen Wall, Scottish Agricultural College, UK
Carol-Anne Duthie, Scottish Agricultural College, UK

***Correspondence:**
Fanny Calenge, INRA, UMR1313 Génétique Animale et Biologie Intégrative, Domaine de Vilvert, 78352 Jouy-en-Josas cedex, France.
e-mail: fanny.calenge@tours.inra.fr

Salmonella enterica serotypes Enteritidis and Typhimurium and *Campylobacter jejuni* are responsible for most cases of food poisoning in Europe. These bacteria do not cause severe disease symptoms in chicken, but they are easily propagated by symptomless chicken carriers which cannot be easily isolated. This animal tolerance is detrimental to food safety. In this particular case, increasing animal's resistance is not sufficient, since some animals considered as resistant are able to carry bacteria during several weeks without displaying disease symptoms. We review studies aimed at evaluating the resistance of chicken to *Salmonella* and *Campylobacter* intestinal colonization, either a few days or several weeks after infection. While studies of the genetic control of *Campylobacter* colonization are only beginning, mostly due to technical difficulties in infection protocols, genetic studies of *Salmonella* colonization have been conducted for now more than 20 years. They have initially reported an estimation of the genetic parameters associated with resistance to *Salmonella* colonization and are now aimed at identifying the genomic regions controlling variation of this trait in experimental lines and commercial populations. With the advent of high-throughput genomics, we are closer than ever to identify the true genes controlling resistance to *Enterobacteria* colonization in chicken. The comparison of genes involved in early resistance to intestinal colonization with genes controlling resistance to bacteria persistence several weeks after infection (i.e., carrier-state) should soon highlight the differences between the molecular mechanisms underlying those two distinct phenotypes. It will also be highly interesting to compare the genes or genomic regions controlling *Campylobacter* and *Salmonella*, in order to evaluate the feasibility of a selection conducted on both bacteria simultaneously.

Keywords: *Salmonella, Campylobacter*, chicken, QTL, genetic architecture, intestinal colonization, carrier-state, candidate gene

INTRODUCTION

According to the most recent EFSA report about food-borne outbreaks in Europe, *Campylobacter*, followed by *Salmonella*, are responsible for most of the reported isolated cases of food-borne diseases, while outbreaks are mostly due to *Salmonella* (EFSA, 2012). These Gram negative *Enterobacteria* live in the intestinal tract of livestock animals (poultry, pigs, and bovine). Bacteria infecting human consumers derive mainly from contaminated avian products, i.e., broiler meat and raw eggs. The main *Salmonella* serotype responsible for human illness, i.e., *Salmonella enterica* serotype Enteritidis, is able to infect broiler chickens or laying hens without causing disease symptoms. Human illness due to *Campylobacter* is mainly due to the species *Campylobacter jejuni*, which is similarly responsible for a silent chicken infection. This animal's ability to carry zoonotic bacteria without showing disease symptoms causes a silent propagation of bacteria in poultry stocks due to the impossibility to isolate contaminated animals. These bacteria are not a threat to animal health but are detrimental to food safety.

Prophylactic measures taken by European countries to clear poultry flocks from *Salmonella* firstly focused on breeder flocks. To prevent vertical transmission, those flocks were systematically checked for absence of contamination by strains of major impact on human health and culled in case of contamination. These procedures have been shown to be efficient (EFSA, 2010) and are now practiced in most flocks. However, they are not sufficient to completely eliminate *Salmonella* Enteritidis and are only efficient in case of vertical propagation, which occurs only for some serotypes of *Salmonella* but not for *Campylobacter*. Genetic selection could be a valuable alternative. The aim of selection in this case would not be to obtain healthy animals since most animals show no disease symptoms, but rather to select more resistant animals with reduced intestinal colonization. In this particular case, animals show an extreme form of tolerance since bacteria colonization is not detrimental to the host health and performance. Simulation studies have shown that using animals more resistant to *Salmonella* intestinal persistence (defined as carrier-state)

in combination with vaccination is indeed efficient to reduce *Salmonella* propagation in laying hen stocks (Prévost et al., 2006, 2008).

As previously reviewed (Calenge et al., 2010), two types of studies related to *Salmonella* intestinal colonization are currently conducted, according to the delay considered after experimental infection, i.e., either a few days or several weeks. Resistance to early *Salmonella* intestinal colonization has been mainly studied at Iowa State University (USA) by a candidate gene approach and at the Institute for Animal Health (IAH, Compton, UK), first by comparison of different chicken lines and more recently by looking for genomic regions controlling intestinal colonization. A similar approach has been undertaken at the National Institute for Agronomical Research (INRA, France) in order to study resistance to bacteria persistence several weeks after infection, defined as resistance to carrier-state.

Resistance to *Campylobacter* intestinal colonization in poultry has been more rarely studied, probably due to technical difficulties for cultivating these anaerobic bacteria and performing reproducible infection tests. The emergence of sanitary concerns about the presence of these bacteria on animal products, especially on broiler carcasses (EFSA, 2012), has reinforced the scientific interest for these bacteria. Only a few studies have already been published, mentioning differences in response to *Campylobacter* infection according to the chicken line tested (Stern et al., 1990; Boyd et al., 2005), which opens the way to genetic selection and to more in-depth genetics studies.

In this paper, we present a review of results obtained on the genetic control of resistance to intestinal colonization by *Campylobacter* and *Salmonella* in fowl. We then discuss the possibility of a partially common genetic control of: (1) resistance to early colonization and to persistence on the one hand, (2) resistance to *Campylobacter* and to *Salmonella* on the other hand. We eventually discuss the scientific opportunities offered by the existence of multiple infection models for the study of *Salmonella* infection, and the necessity of an integrative genomics approach to better understand the genetic control of resistance to *Enterobacteria* carrier-state.

GENETIC CONTROL OF RESISTANCE TO *Salmonella* INTESTINAL COLONIZATION IN CHICKEN

In the 1980s, researchers and breeders began to take an interest in the serotypes responsible for human cases of salmonellosis, i.e., *S.* Enteritidis and *S.* Typhimurium, while previous scientific studies had been focusing on species specific serotypes (*S.* Gallinarum, *S.* Pullorum) causing acute salmonellosis in chickens (Wigley, 2004; Calenge et al., 2010). Different infections models have been used to evaluate resistance to intestinal colonization by these serotypes (Calenge et al., 2010). The main differences between these models are the age at which experimental infections are carried out (either young chicks/hatchlings or adult laying hens), the age at which the level of colonization is measured (a few days or several weeks p.i.) and the way intestinal colonization is measured (cecal load or fecal shedding). Studying very young chicks is essential since commercial broilers are often infected at a very young age. On the other hand, bacteria excretion is a great concern for laying hens when hens reach the laying peak, since bacteria can easily contaminate egg shells. To evaluate intestinal colonization, bacteria are counted in ceca, which is a reservoir for intestinal bacteria, or in feces. The level of intestinal colonization measured a few days after infection evaluates the *Salmonella* shedding potential of each bird immediately after infection. Nevertheless, it does not allow an estimation of persistent shedding, which can only be evaluated several weeks after infection.

A series of publications investigated the role of candidate genes otherwise known for their role in immunity in the observed variability of cecal load, 1 week after infection of 1-day old chicks (Calenge et al., 2010). Several genes, namely *CD28*, *IAP1*, *TGF-*$\beta_{2,3,4}$, *Gal*$_{11,12,13}$, *TRAIL*, *IL*$_{-2,10}$, *PSAP*, *SLC11A1*, *IGL*, *CASP1*, *iNOS*, *PIGR,* and *MAPKAPK12* were actually associated with variation for cecal load of *S.* Enteritidis (Kaiser et al., 1998, 2002; Kaiser and Lamont, 2001, 2002; Lamont et al., 2002; Liu et al., 2002, 2003; Kramer et al., 2003; Malek and Lamont, 2003; Malek et al., 2004; Hasenstein et al., 2006). The effects of the candidate genes *SLC11A1* and *TLR4* have been largely studied in several experimental populations (Calenge et al., 2010). Nevertheless none of those genes had a major effect. To complete these studies, the effects of these genes should be studied in other populations in order to evaluate their stability and importance in the control of *Salmonella* intestinal colonization. This has been done for *SLC11A1* and *TLR4* in several independent studies, which failed to identify major and stable effects of these genes.

To study *S.* Enteritidis persistence, two models of infection were developed at INRA, differing in the age at which animals are infected: at 1 week of age (Duchet-Suchaux et al., 1995, 1997) or at the laying peak (Protais et al., 1996). Conditions were chosen with which all animals are carrying bacteria shortly after infection but are able to get rid of them in a few weeks (Duchet-Suchaux et al., 1995; Protais et al., 1996). Measures are made several weeks after infection in order to evaluate the animal's ability to completely clear pathogenic *Salmonella* from its digestive tract. After studies conducted to estimate the heritability of resistance to carrier-state (Girard-Santosuosso et al., 1998, 2002), a divergent selection experiment was conducted on commercial laying hens (Beaumont et al., 2009). Interestingly, this experiment showed that genetic resistance at a young age was negatively correlated to adult resistance. In other words, some genes contributing to carrier-state resistance at a young age have an antagonistic effect on adult animals. This could be related to the immaturity of the immune system in chicks, which implies that some of the genes controlling resistance to carrier-state could be involved in the immune response.

In order to identify the genomic regions controlling resistance to *S.* Enteritidis intestinal carrier-state, QTL analyses experiments were then carried out using experimental White Leghorn Inbred lines. These lines had been shown to display different levels of resistance to different serotypes of *Salmonella* (Bumstead and Barrow, 1988, 1993; Bumstead et al., 1991). The first analysis was a selective genotyping approach using a F2 progeny derived

from parental inbred lines N and 6_1, conducted only on animals displaying extreme phenotypes (Tilquin et al., 2005). It was followed by a confirmation study after genotyping the whole F2 progeny (Calenge et al., 2009). Both studies used microsatellite genotypings. The two most significant QTLs were identified and confirmed on chromosomes 2 and 16. Interestingly, the QTL on chromosome 16 is located on the Major Histocompatibility Complex, so that one of the genes belonging to this complex is probably the actual gene at the QTL. A following analysis was performed with a more complete and denser genome scan using 480 highly informative SNP markers and a higher number of animals. It led to several QTLs on previously uncovered microchromosomes but failed to confirm the major QTL on chromosome 2, while the effect of the QTL located on the MHC on chromosome 16 could not be confirmed due to the absence of segregating SNP markers in this genome region (Calenge et al., 2011). To test the influence of the detection method on the QTL identified, an additional analysis was performed using maximum likelihood, whereas previous studies used linear regression. With the maximum likelihood method developed in the MapQTL software, the possibility of gene segregation within the parental lines could be taken into account. Intriguingly, although phenotypic and genotypic data were identical, QTL were completely different (Tran et al., 2012). This apparent discrepancy is probably a consequence of the different hypotheses underlying both calculation methods, which have a greater impact on QTL with weak effects. In addition, dominance effects could not be taken into account with the maximum likelihood method used, so that all QTL with a strong dominant effect could not be detected. In parallel, a similar QTL analysis of *Salmonella* early intestinal colonization has been carried out at IAH using a distinct infection model in which animals were evaluated a short time after infection (Fife et al., 2011). Interestingly, two of the four QTL detected are located close to QTL controlling *S.* Enteritidis persistence, so that it can be speculated that these QTL have pleiotropic effects both on *S.* Typhimurium early colonization and on *S.* Enteritidis persistence (Tran et al., 2012).

On the whole, these candidate gene and QTL analysis studies show a complex control of *Salmonella* intestinal colonization in laying hens, with many QTL or candidate genes having weak effects varying according to animal's age, parental lines, and also QTL detection method. The detection of one QTL on the MHC and the influence if animal's age on QTL detection lead us to the hypothesis that some of the genes controlling carrier-state are involved in the immune response. This would be coherent with the assumption that a better resistance to *Salmonella* early colonization is one of the mechanisms leading to better resistance to carrier-state. At this stage, although some of the QTLs identified have been validated in commercial lines (Calenge et al., 2009), marker assisted selection is not possible because of the small effects of QTLs and of their large confidence intervals.

A COMMON GENETIC CONTROL FOR RESISTANCE TO *Salmonella* AND *Campylobacter* CARRIER-STATE?

Campylobacter and *Salmonella* are both Gram negative *Enterobacteria* living in the host intestine, silently carried by chickens and causing gastro-intestinal disease in humans. For these reasons, both are a concern for food safety rather than for animal health. These similarities naturally lead to the conclusion that the genetic control of carrier-state could be at least partly common for these bacteria. Only a few studies have been published about the genetic control of *Campylobacter* resistance. A first study in 1990 showed genetic differences between caecal loads of three commercial broiler lines (Stern et al., 1990). It was followed by a comparison of several White Leghorn inbred layer lines, which showed significant differences in the number of bacteria in the caeca or cloaca between the lines studied (Boyd et al., 2005). Another study demonstrated differences in *C. jejuni* cecal colonization between two different broiler lines (Li et al., 2008). Interestingly, the same inbred layer lines N and 6 that display different levels of resistance to *Salmonella* carrier-state showed different levels of resistance to *Campylobacter* colonization, which strengthens the hypothesis of a common genetic control of both bacteria (Boyd et al., 2005). At INRA a first, preliminary comparison of different chicken lines for their resistance level to *C. jejuni* carrier-state was conducted. It included the N and 6 lines, with a different infection model developed at ANSES (Ploufragan, France). Nevertheless it did not reveal significant differences in carrier-state levels between the lines studied, with the exception of Fayoumi which showed a lower level of *C. jejuni* carrier-state. This shows the great influence of the infection protocol on the results observed. A more recent gene expression study of the local cecal response to *Campylobacter* colonization mentions two broiler lines differing for their susceptibility to *Campylobacter* (Li et al., 2008, 2010). This study identified distinct transcriptional profiles between both lines, with genes identified for the first time in avian infection studies (Li et al., 2010). These results strengthen the hypothesis of a genetic control of resistance to *Campylobacter* and also tends to favor the hypothesis of genetic control specific to this bacterial species.

A recent QTL analysis of *Campylobacter* colonization was performed in a progeny derived from lines N and 6 by using the infection protocol developed by Boyd et al. (2005). Four QTL with locations independent from those of the QTL for *Salmonella* colonization were identified in a similar progeny, i.e., a backcross population [6 × N] × N (Kaiser, 2010). The author concluded to the absence of common resistance genes for both bacteria, which is not so surprising, when citing the author, considering that *Campylobacter* and *Salmonella* infections differ in their physiopathology and in the innate immune responses involved (Shaughnessy et al., 2009; Kaiser, 2010). Nevertheless, *Campylobacter* QTL locations were compared only with those of QTL for resistance to *Salmonella* colonization, and not with those of QTL for *Salmonella* carrier-state. It appears that three of the QTL identified, on chromosomes 7, 11, and 27, co-localize (i.e., their confidence intervals overlap) with QTL for resistance to carrier-state (Tilquin et al., 2005; Calenge et al., 2009, 2011). Therefore, although there is probably no unique genetic resistance control for both bacteria, some genes could be common when considering carrier-state and not only early colonization. It would be much interesting to know ultimately in which part

of the immune resistance mechanisms those common genes are involved: innate or acquired resistance, tolerance mechanisms, etc. In short, chicken line comparison studies available are apparently contradictory, probably due to differences in the infection protocols used, while comparison of QTL analyses points to QTL co-locations between resistance to *Salmonella* carrier-state and resistance to *Campylobacter* colonization. These results show the absence of an obvious common genetic determinism but do not discard the possibility of a few common genes. Further research is needed to better understand the genetic architecture of resistance to *Campylobacter* carrier-state, with much attention paid to the infection protocol used, since different protocols can lead to opposite conclusions.

Future studies should also take into account the host intestinal microbiota, since recent research conducted both on human and livestock demonstrates the previously underestimated impact of this microbiota on the host ability to mount an immune response and to control pathogens (Kosiewicz et al., 2011). Interactions between gut microbiota and immune system have already been demonstrated in chicken (Brisbin et al., 2008). The role of microbiota in the establishment of an immune response after *S.* Enteritidis has already been questioned (Crhanova et al., 2011) and the microbiota response to a challenge by *C. jejuni* has been studied (Qu et al., 2008). In order to colonize host intestines, pathogenic *Enterobacteria* must overcome the resistance mediated by the gut microbiota and the innate immune system. While some studies conclude to the absence of effect of *Salmonella* or *Campylobacter* colonization on host microbiota composition (Qu et al., 2008; Nordentoft et al., 2011), others mention effects of *S.* Enteritidis colonization on the gut immune response when compared to normal microbiota (Crhanova et al., 2011). If microbiota composition does not change following *Campylobacter* or *Salmonella* colonization, which should be confirmed in other studies, it does not preclude any change in functional interactions between microbiota and host immune response. This area of research is worth being further explored. If feasible, influencing microbiota composition through host genetic selection or nutrition could be an indirect way to limit tolerance to intestinal pathogens. A recent study using mouse advanced intercross lines (AIL) has demonstrated the role of host genetics control in shaping individual microbiome diversity (Benson et al., 2010). Authors define a core measurable microbiota which variations are under host genetic control. It would be particularly interesting to know if host genes determine microbiota composition in chicken and if those genes have an indirect impact on pathogenic *Enterobacteria* colonization and carrier-state.

MANY INFECTION PROTOCOLS AND PHENOTYPES TO STUDY *Salmonella* INFECTION: WEAKNESS OR STRENGTH?

Since QTL for *Salmonella* carrier-state identified are relatively unstable according to many parameters (chicken age, calculation method), to strengthen our results we turned to other studies conducted using other *Salmonella* infection protocols. The many differences in the infection protocols used to study *Salmonella* resistance or carrier-state render comparisons of results difficult, since protocols differ in many ways and it is impossible to decipher which condition exactly led to different results (Calenge et al., 2010). Nevertheless, the reliability and interest of QTL are strengthened when QTL detected using two different infection protocols and co-localizing. Co-location can provide some hints on the possible way of action of co-localizing QTLs, although with the great size of QTL confidence intervals, these considerations are speculative and have to be confirmed by more in-depth studies. This is what led us to underline the interest of QTLs identified on chromosomes 2 and 3, which were also identified in independent studies of *Salmonella* Typhimurium early colonization in the 6_1 and 15I White Leghorn inbred lines (Fife et al., 2011). Using SNP markers located close to these QTL (Fife et al., 2011), QTL detection was slightly improved (Tran et al., 2012). One or several genes controlling early colonization to *Salmonella* could thus very well be involved in the control of *Salmonella* carrier-state. Another example illustrating the interest of comparing QTL locations from independent studies interested in different phenotypes is the study of Redmond et al. (2011), compared to results of Fife et al. (2009). It appears that SNP markers associated with heterophil function were identified very close to the gene *SIVA1*, candidate for the major QTL *SAL1*, involved in the control of splenic *S.* Typhimurium load (Mariani et al., 2001; Fife et al., 2009). Since *SIVA1* is a likely regulator of heterophil function, its co-location with SNP markers involved in heterophil function strengthens the plausibility of its causal role for the *SAL1* major QTL (Redmond et al., 2011). The other interest of this study is the great precision of the phenotype assessed, which gives access to possible gene functions. A finer phenotyping of resistance taking into account all levels of host reaction to invading pathogens, i.e., from disease symptoms to the molecules and cells involved in innate or adaptive immune response, through the composition of host gut microbiota and the intestinal immune response, should be considered as an interesting strategy to characterize the functions of QTLs and strengthen plausible positional candidate genes. These examples of QTL co-location show that the existence of many different *Salmonella* infection protocols can be seen as strength to characterize QTL functions rather than a weakness preventing comparisons. Ideally, QTL or gene locations should be compared on the same animal material. When truly causal genes will be eventually identified, the existence of multiple infection models will enable researchers to understand the genetic origin of differences between early colonization and carrier-state, but also between *Salmonella* and *Campylobacter* infection.

TOWARD CAUSAL GENES IDENTIFICATION: NECESSITY OF AN INTEGRATIVE APPROACH

The identification of the causal genes underlying QTL for resistance to carrier-state and to colonization would be a great progress toward a better understanding of the mechanisms differentiating *Enterobacteria* true resistance and carrier-state. Are causal genes involved in the innate or adaptive immune response or key regulators genes controlling several metabolic pathways? Are they directly or indirectly responsible for a shift in gut microbiota composition or involved in mechanisms circumventing or escaping immune resistance mechanisms? Those questions will

be answered only when causal genes will be identified. Until now at least, classical QTL analyses have found their limits. QTL confidence intervals are too vast to reasonably point to one or several candidate genes, with the only exception of the major QTL *SAL1* (Mariani et al., 2001), which phenotypic effects were important enough to allow classical genetics studies to identify only a few candidate genes (Fife et al., 2009). Two striking candidate genes were proposed for this QTL: *SIVA1*, coding for the CD27-binding protein Siva and *AKT1*, coding for the RAC-alpha serine/threonine protein kinase homolog (Fife et al., 2009). More generally, before choosing candidate genes, QTL locations need to be refined. AIL are a material of choice to reach this purpose (Darvasi and Soller, 1995). They have already been successfully used in chicken to refine QTL for body weight (Besnier et al., 2011) or QTL affecting resistance to Marek's disease (Heifetz et al., 2009). Thanks to the advent of high-throughput genotyping, their high rate of recombinations can now easily be exploited to fine map QTLs. Interestingly, an independent study confirmed *SIVA1* as most probable candidate for *SAL1* by looking for SNP markers associated with heterophil function in AIL of chicken (Redmond et al., 2011). The latter study identified SNP markers associated with heterophils extra-cellular trap (HET) production, thus indicating a possible role for *SIVA1* as a regulator of HET production (Redmond et al., 2011). This study well demonstrates the interest of coupling QTL fine-mapping strategies with high density genotyping to reduce QTL confidence intervals. It is probable that this strategy was successful with *SAL1* due to the importance of its effect on splenic *Salmonella* colonization. It can be questioned whether the exploitation of AIL will be fruitful for QTL with much weaker effect: QTL confidence intervals will probably be refined, but not to a single or even a few candidate(s), unless very striking candidate genes appear.

To reach causative genes at QTLs, it seems relevant to conduct an integrative approach by leading several types of analyses simultaneously, in order both to cross-validate QTL locations and to characterize their functions. Comparing QTL locations identified in independent studies is interesting. Although it does not lead to causal genes, it can give hints regarding their function by linking different phenotypes. This was done to confirm the probable role of *SAL1* in regulating heterophil function (Redmond et al., 2011). Candidate gene approaches, taken alone, are not sufficient to explain the totality of phenotype variations, but when candidate genes co-localize with QTL they become even more interesting. This is why the involvement of functional candidate genes, annotated to be involved in the immune response or differentially expressed between parental lines, could be more systematically investigated. Many expression studies before/after challenge with pathogenic *Enterobacteria* have been conducted, sometimes with different chicken lines, and their results could be better taken into account (Calenge et al., 2010). Before the availability of the chicken genome sequence, candidate gene approaches have successfully been conducted in chicken to study resistance to *Salmonella* carrier-state. The roles of the genes *SLC11A1* (previously named *NRAMP1*) and *TLR4* have been studied in several chicken lines (Girard-Santosuosso et al., 2002; Lamont et al., 2002; Beaumont et al., 2003; Kramer et al., 2003; Leveque et al., 2003; Calenge et al., 2009). Many gene related to the immune response have also been the object of focused studies (Lamont et al., 2002; Liu et al., 2002; Kramer et al., 2003; Malek and Lamont, 2003; Malek et al., 2004; Hasenstein et al., 2006; Hasenstein and Lamont, 2007; Ghebremicael et al., 2008). This candidate gene approach only led to the detection of slight effects, which is coherent with what was observed for QTL analyses, with many QTL of small to medium effect. From these two different approaches it appears that resistance to *Salmonella* carrier-state is apparently controlled by several genes of small effect, probably varying according to chicken breed, chicken age, parameters related to the infection protocol used (i.e., inoculum dose, time post infection, etc.). This is most probably the case for resistance to other *Enterobacteria*. Furthermore, a recent study demonstrates the role of epigenetic regulation of TLR gene expression in the resistance to *S.* Enteritidis colonization (Gou et al., 2012). It would be much interesting to know whether those epigenetic modifications are under host genetic control.

CONCLUSION

Integrative studies allowed by the advent of high-throughput genomics should soon lead to causal genes for *Enterobacteria* intestinal colonization in chicken. Nevertheless, even in the most favorable case in which we know several causal genes, genetic selection will be complicated by their weak to medium effect and by their instability according to chicken line, age and environment. In addition, intestinal colonization by *Campylobacter* and *Salmonella* are probably not controlled by the same genes. In this context, could genomic selection be considered as the obvious solution for commercial selection of chicken more resistant to bacteria intestinal colonization? It seems promising, since SNP markers causing variation for the trait considered are directly selected within the selection stock without looking for causal genes (Goddard et al., 2010), thus preventing the need for checking QTL or gene stability according to many parameters. Indeed, a first study gave interesting results for SNP-assisted selection for resistance to *Salmonella* carrier-state in laying hens (Legarra et al., 2011). Nevertheless, for the time being many obstacles stand in the way of genomic selection for disease resistance in chicken. One of them is the necessity to challenge, before any application and repeatedly during the selection process, a very high number (several thousands) of animals belonging to the reference population. This seems hardly feasible for the study of *Salmonella* or *Campylobacter* intestinal colonization, which implies to count bacteria in caeca or spleen, even if both diseases have a high economic and social impact and can thus be considered as traits of interest for commercial application (Davies et al., 2009). Alternatively, integrative genomics approaches combined with recent dramatic advances in genotyping costs and efficiency should soon lead to the identification of causal genes at the QTL, thus precluding the need for recurrent test of the association of causal genes with disease resistance and leading to much more accurate and reliable genomic assessment.

REFERENCES

Beaumont, C., Chapuis, H., Protais, J., Sellier, N., Menanteau, P., Fravalo, P., et al. (2009). Resistance to Salmonella carrier state: selection may be efficient but response depends on animal's age. *Genet. Res.* 91, 161–169.

Beaumont, C., Dambrine, G., Chaussé, A.-M., and Flock, D. (2003). "Selection for disease resistance: conventional breeding for resistance to bacteria and viruses," in *Poultry Genetics, Breeding and Biotechnology*, eds W. Muir and S. Aggrey (Wallingford, UK: CAB International), 357–384.

Benson, A., Kelly, S., Legge, R., Ma, F., Low, S., Kim, J., et al. (2010). Individuality in gut microbiota composition is a complex polygenic trait shaped by multiple environmental and host genetic factors. *Proc. Natl. Acad. Sci. U.S.A.* 107, 18933–18938.

Besnier, F., Wahlberg, P., Rönnegård, L., Ek, W., Andersson, L., Siegel, P., et al. (2011). Fine mapping and replication of QTL in outbred chicken advanced intercross lines. *Genet. Sel. Evol.* 17, 3.

Boyd, Y., Herbert, E., Marston, K., Jones, M., and Barrow, P. (2005). Host genes affect intestinal colonisation of newly hatched chickens by *Campylobacter jejuni*. *Immunogenetics* 57, 248–253.

Brisbin, J., Gong, J., and Sharif, S. (2008). Interactions between commensal bacteria and the gut-associated immune system of the chicken. *Anim. Health Res. Rev.* 9, 101–110.

Bumstead, N., and Barrow, P. (1988). Genetics of resistance to *Salmonella typhimurium* in newly hatched chicks. *Br. Poult. Sci.* 29, 521–529.

Bumstead, N., and Barrow, P. (1993). Resistance to *Salmonella gallinarum*, *S. pullorum and S. enteritidis* in inbred lines of chickens. *Avian Dis.* 37, 189–193.

Bumstead, N., Millard, B., Barrow, P., and Cook, J. (1991). "Genetic basis of disease resistance in chickens," in *Breeding for Disease Resistance in Farm Animals,* eds J. Owen and R. Axford (Wallingford, UK: CAB International), 10–23.

Calenge, F., Kaiser, P., Vignal, A., and Beaumont, C. (2010). Genetic control of resistance to salmonellosis and to Salmonella carrier-state in fowl: a review. *Genet. Sel. Evol.* 42, 11.

Calenge, F., Lecerf, F., Demars, J., Feve, K., Vignoles, F., Pitel, F., et al. (2009). QTL for resistance to Salmonella carrier state confirmed in both experimental and commercial chicken lines. *Anim. Genet.* 40, 590–597.

Calenge, F., Vignal, A., Demars, J., Fève, K., Menanteau, P., Velge, P., et al. (2011). New QTL for resistance to Salmonella carrier-state identified on fowl microchromosomes. *Mol. Genet. Genomics* 285, 237–243.

Crhanova, M., Hradecka, H., Faldynova, M., Matulova, M., Havlickova, H., Sisak, F., et al. (2011). Immune response of chicken gut to natural colonization by gut microflora and to *Salmonella enterica* serovar enteritidis infection. *Infect. Immun.* 79, 2755–2763.

Darvasi, A., and Soller, M. (1995). Advanced intercross lines, an experimental population for fine genetic mapping. *Genetics* 141, 1199–1207.

Davies, G., Genini, S., Bishop, S., and Giuffra, E. (2009). An assessment of opportunities to dissct host genetic variation in resistance to infectious diseases in livestock. *Anim. Genet.* 3, 415–436.

Duchet-Suchaux, M., Léchopier, P., Marly, J., Bernardet, P., Delaunay, R., and Pardon, P. (1995). Quantification of experimental *Salmonella enteritidis* carrier state in B13 leghorn chicks. *Avian Dis.* 39, 796–803.

Duchet-Suchaux, M., Mompart, F., Berthelot, F., Beaumont, C., Léchopier, P., and Pardon, P. (1997). Differences in frequency, level and duration of cecal carriage between four outbred chicken lines infected orally with *Salmonella enteritidis*. *Avian Dis.* 41, 559–567.

EFSA. (2010). The community summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in the European union in 2008. *EFSA J.* 288. doi: 10.2903/j.efsa.2009.1438

EFSA. (2012). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2010. *EFSA J.* 10, 2597.

Fife, M., Howell, J., Salmon, N., Hocking, P., Van Diemen, P., Jones, M., et al. (2011). Genome-wide SNP analysis identified major QTL for *Salmonella* colonization in the chicken. *Anim. Genet.* 42, 134–140.

Fife, M., Salmon, N., Hocking, P., and Kaiser, P. (2009). Fine mapping of the chicken salmonellosis resistance locus (SAL1). *Anim. Genet.* 40, 871–877.

Ghebremicael, S., Hasenstein, J., and Lamont, S. (2008). Association of interleukin-10 cluster genes and *Salmonella* response in the chicken. *Poult. Sci.* 87, 22–26.

Girard-Santosuosso, O., Lantier, F., Lantier, I., Bumstead, N., Elsen, J.-M., and Beaumont, C. (2002). Heritability of susceptbility to *Salmonella enteritidis* infection in fowls and test of the role of the chromosome carrying the NRAMP1 gene. *Genet. Sel. Evol.* 342, 211–219.

Girard-Santosuosso, O., Menanteau, P., Duchet-Suchaux, M., Berthelot, F., Mompart, F., Protais, J., et al. (1998). Variability in the resistance of four chicken lines to experimental intravenous infection with *Salmonella enteritidis* phage type 4. *Avian Dis.* 42, 462–469.

Goddard, M., Hayes, B., and Meuwissen, T. (2010). Genomic selection in livestock populations. *Genet. Res.* 92, 413–421.

Gou, Z., Liu, R., Zhao, G., Zheng, M., Li, P., Wang, H., et al. (2012). Epigenetic modification of TLRs in leukocytes is associated with increased susceptibility to *Salmonella enteritidis* in chickens. *PLoS ONE* 7:e33627. doi: 10.1371/journal.pone.0033627

Hasenstein, J., and Lamont, S. (2007). Chicken gallinacin gene cluster associated with Salmonella response in advanced intercross line. *Avian Dis.* 51, 561–567.

Hasenstein, J., Zhang, G., and Lamont, S. (2006). Analyses of five gallinacin genes and the *Salmonella enterica* serovar Enteritidis response in poultry. *Infect. Immun.* 74, 3375–3380.

Heifetz, E., Fulton, J., O'Sullivan, N., Arthur, J., Cheng, H., Wang, J., et al. (2009). Mapping QTL affecting resistance to Marek's disease in an F6 advanced intercross population of commercial layer chickens. *BMC Genomics* 10:20. doi: 10.1186/1471-2164-10-20

Kaiser, M., and Lamont, S. (2001). Genetic line differences in survival and pathogen load in young layer chicks after *Salmonella enterica* serovar Enteritidis exposure. *Poult. Sci.* 80, 1005–1108.

Kaiser, M., Wing, T., and Lamont, S. (1998). Effects of genetics, vaccine dosage, and postvaccination sampling interval on early antibody response to *Salmonella enteritidis* vaccine in broiler breeder chicks. *Poult. Sci.* 77, 271–275.

Kaiser, M. G., Lakshmanan, N., Wing, T., and Lamont, S. J. (2002). *Salmonella enterica* serovar enteritidis burden in broiler breeder chicks genetically associated with vaccine antibody response. *Avian Dis.* 46, 25–31.

Kaiser, M. G., and Lamont, S. F. (2002). Microsatellites linked to *Salmonella enterica* serovar enteritidis burden in spleen and Cecal content of young F-1 broiler-cross chicks. *Poult. Sci.* 81, 657–663.

Kaiser, P. (2010). Advances in avian immunology – prospects for disease control: a review. *Avian pathol.* 39, 309–324.

Kosiewicz, M., Zirnheld, A., and Alard, P. (2011). Gut microbiota, immunity, and disease: a complex relationship. *Front. Microbiol.* 2:180. doi: 10.3389/fmicb.2011.00180

Kramer, J., Malek, M., and Lamont, S. (2003). Association of twelve candidate gene polymorphisms and response to challenge with *Salmonella enteritidis* in poultry. *Anim. Genet.* 34, 339–348.

Lamont, S., Kaiser, M., and Liu, W. (2002). Candidate genes for resistance to *Salmonella enteritidis* colonization in chickens as detected in a novel genetic cross. *Vet. Immunol. Immunopathol.* 87, 423–428.

Legarra, A., Calenge, F., Mariani, P., Velge, P., and Beaumont, C. (2011). Use of a reduced set of SNP for genetic evaluation of resistance to cvarrier state in laying hens. *Poult. Sci.* 90, 731–736.

Leveque, G., Forgetta, V., Morroll, S., Smith, A., Bumstead, N., Barrow, P., et al. (2003). Allelic variation in *TLR4* is linked to susceptibility to *Salmonella enterica* serovar typhimurium infection in chcickens. *Infect. Immun.* 71, 1116–1124.

Li, X., Swaggerty, C., Kogut, M., Chiang, H., Wang, Y., Genovese, K., et al. (2008). The paternal effect of *Campylobacter jejuni* colonization in ceca in broilers. *Poult. Sci.* 87, 1742–1747.

Li, X., Swaggerty, C., Kogut, M., Chiang, H.-I., Wang, Y., Genovese, K., et al. (2010). Gene expression profiling of the local response of genetic chicken lines that differ in their susceptibility to *Campylobacter jejuni* colonization. *PLoS ONE* 5:e11827. doi: 10.1371/journal.pone.0011827

Liu, W., Kaiser, M., and Lamont, S. (2003). Natural resistance-associated macrophage protein 1 gene polymorphisms and response to vaccine against or challenge with *Salmonella enteritidis* in young chicks. *Poult. Sci.* 82, 259–266.

Liu, W., Miller, M., and Lamont, S. (2002). Association of MHC class I and class I gene polymorphisms with vaccine or challenge response to *Salmonella enteritidis* in

young chicks. *Immunogenetics* 54, 582–590.

Malek, M., Hasenstein, J., and Lamont, S. (2004). Analysis of chicken TLR4, CD28, MIF, MD-2, and LITAF genes in a *Salmonella enteritidis* resource population. *Poult. Sci.* 83, 544–549.

Malek, M., and Lamont, S. (2003). Association of *INOS*, *TRAIL*, *TGF-beta2*, *TGF-beta3*, and *IGL* genes with response to *Salmonella enteritidis* in poultry. *Genet. Sel. Evol.* 35, S99–S111.

Mariani, P., Barrow, P., Chang, H., Groenen, M., Negrini, R., and Bumstead, N. (2001). Localization to chicken chromosome 5 of a novel locus determining salmonellosis resistance. *Immunogenetics* 53, 786–791.

Nordentoft, S., Mølbak, L., Bjerrum, L., De Vylder, J., Van Immerseel, F., and Pedersen, K. (2011). The influence of the cage system and colonisation of *Salmonella enteritidis* on the microbial gut flora of laying hens studied by T-RFLP and 454 pyrosequencing. *BMC Microbiol.* 11:187. doi: 10.1186/1471-2180-11-187

Prévost, K., Magal, P., and Beaumont, C. (2006). A model of Salmonella infection within industrial house hens. *J. Theor. Biol.* 242, 755–763.

Prévost, K., Magal, P., Protais, J., and Beaumont, C. (2008). Effect of genetic resistance of the hen to Salmonella carrier-state on incidence of bacterial contamination: synergy with vaccination. *Vet. Res.* 39, 20.

Protais, J., Colin, P., Beaumont, C., Guillot, J., Lantier, F., Pardon, P., et al. (1996). Line differences in resistance to *Salmonella enteritidis* PT4 infection. *Br. Poult. Sci.* 37, 329–339.

Qu, A., Brulc, J., Wilson, M., Law, B., Theoret, J., Joens, L., et al. (2008). Comparative metagenomics reveals host specific metavirulomes and horizontal gene transfer elements in the chicken cecum microbiome. *PLoS ONE* 13:e2945. doi: 10.1371/journal.pone.0002945

Redmond, S., Chuammitri, P., Andreasen, C., Palić, D., and Lamont, S. (2011). Genetic control of chicken heterophil function in advanced intercross lines: associations with novel and with known Salmonella resistance loci and a likely mechanism for cell death in extracellular trap production. *Immunogenetics* 63, 449–458.

Shaughnessy, R., Meade, K., Cahalane, S., Allan, B., Reiman, C., Callanan, J., et al. (2009). Innate immune gene expression differentiates the early avian intestinal response between Salmonella and Campylobacter. *Vet. Immunol. Immunopathol.* 132, 191–198.

Stern, N., Meinersmann, R., Cox, N., Bailey, J., and Blankenship, L. (1990). Influence of host lineage on cecal colonization by *Campylobacter jejuni* in chickens. *Avian Dis.* 34, 602–606.

Tilquin, P., Barrow, P., Marly, J., Pitel, F., Plisson-Petit, F., Velge, P., et al. (2005). A genome scan for quantitative trait loci affecting the Salmonella carrier-state in the chicken. *Genet. Sel. Evol.* 37, 539–561.

Tran, T.-S., Beaumont, C., Salmon, N., Fife, M., Kaiser, P., Le Bihan-Duval, E., et al. (2012). A maximum likelihood QTL analysis reveals common genome regions controlling resistance to Salmonella colonization and carrier-state. *BMC Genomics* 13:198. doi: 10.1186/1471-2164-13-198

Wigley, P. (2004). Genetic resistance to Salmonella infection in domestic animals. *Res. Vet. Sci.* 76, 165–169.

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A mathematical model to study resistance and tolerance to infection at the animal and population levels: application to *E. coli* mastitis

Johann C. Detilleux*

Quantitative Genetics Group, Faculty of Veterinary Medicine, University of Liège, Liège, Belgium

Edited by:
Andrea B. Doeschl-Wilson, The University of Edinburgh, UK

Reviewed by:
Vinca Russell, University of Edinburgh, UK
Beatriz Villanueva, INIA, Spain
Liesbeth Van Der Waaij, Wageningen University, Netherlands

***Correspondence:**
Johann C. Detilleux, Quantitative Genetics Group, Faculty of Veterinary Medicine, University of Liège, Liège, Belgium.
e-mail: jdetilleux@ulg.ac.be

A mathematical model is proposed that describes the colonization of host tissues by a contagious pathogen and the early nonspecific immune response, the impact of the infection on the performances of the host, and the spread of the infection in the population. The model obeys specific biological characteristics: Susceptible hosts are infected after contact with an infected one. The number of pathogenic units that invade a susceptible host is dependent on the infectious dose provided by the infected host and on the ability of the susceptible host to resist the invasion. After entry in host, pathogenic changes over time are expressed as the difference between the intrinsic logistic growth rate and the Holling type II kill rate provided by the immune response cells. Hosts have different ability to restrict reproduction of the pathogen units. The number of response cells actively recruited to the site of infection depends on the number of the pathogenic units. Response cells are removed after having killed a fixed number of pathogenic units. The effects of the number of pathogenic units on the performances of the host depend upon its levels of tolerance to the deleterious effects of both pathogenic and response cells. Pre-infection costs are associated to tolerance and resistance levels. Estimates of most biological parameters of the model are based on published experimental studies while resistance/tolerance parameters are varied across their allowable ranges. The model reproduces qualitatively realistic outcomes in response to infection: healthy response, recurrent infection, persistent infectious and non-infectious inflammation, and severe immunodeficiency. Evolution across time at the animal and population levels is presented. Effects on animal performances are discussed with respect to changes in resistance/tolerance parameters and selection strategies are suggested.

Keywords: resistance, tolerance, infection, mathematics

INTRODUCTION

Many conservation and selection programs (e.g., FAO, 2007; Eadgene, 2012) include increasing ability to fight endemic disease as an objective. The first challenge to meet this objective is to accurately define and measure disease resistance and tolerance.

Resistance traits are broadly defined as host traits that reduce the extent of pathogen infection. They include traits that reduce pathogen transmission at contact and pathogen growth rate once infection has occurred (Kover and Schaal, 2002). Controlled immune response is a major mediator of resistance because of its efficacy in clearing infections (Sears et al., 2011). Operationally, resistance is typically measured as the inverse of infection intensity (number of parasites per host or per unit host tissue) and a lower intensity means an animal is more resistant, all else being equal (Råberg et al., 2009; Medzhitov et al., 2012).

Tolerance, on the other hand, is defined as the host's ability to reduce the effect of infection on its fitness. Although fitness measurements include different life-history traits, only performance (e.g., growth, milk or wool) is considered as it is very important in farm animals. Tolerance may be targeted to reduce damage directly inflicted by the pathogen (direct tolerance) or caused by the immune response (indirect tolerance). Little is known about underlying mechanisms of tolerance (see one example in Medzhitov, 2009) although they potentially include tissue repair and immunological mechanisms (Råberg et al., 2007). Tolerance is usually operationally defined as the slope of a regression of host performance against infection intensity and a steeper the slope means lower tolerance (Råberg et al., 2009; Medzhitov et al., 2012).

Costs are associated with both resistance and tolerance because energy is required to maintain immune-competence and to mount an efficient immune response, as shown in various empirical studies (Boots and Bowers, 1999; Canale and Henry, 2010). Microarray analyses of the early response to infection with mammary pathogens have also revealed reorganization of gene expression involved in energy metabolism (Bonnefont et al., 2012). If energy is required to uphold resistance and tolerance, less is available to maintain fitness (resource allocation theory; Oltenacu and Algers, 2005). So, authors have proposed to measure these resource allocation costs by comparing performances of resistant and tolerant hosts in pathogen-free environments (e.g., Nunez-Farfan et al., 2007; Rohr et al., 2010).

Unfortunately, levels and costs associated to resistance and tolerance are usually difficult to obtain in field studies under pathogen attack. Given these technical difficulties, their relative importance is here investigated using mathematical simulation studies. Hence, the main objective of the paper is to investigate and the effects of resistance and tolerance on the spread of an infectious disease and on the performances of the animals within a closed population, for a range of realistic scenarios. At the animal level, a comprehensive model is constructed that incorporates important biological characteristics associated with the early immune response to infection.

MATERIALS AND METHODS

The model has two main components, each with two parts. The first system of equations describes the changes in cell concentrations associated with the infection. The second expresses the effects of the infection on host performances. Both are made stochastic rather than deterministic to capture the variability inherent in biological processes.

SYSTEM OF EQUATIONS FOR PATHOGEN AND IMMUNE CELLS DYNAMICS

The system of equations elaborates on a previous discrete susceptible–infected–susceptible model (Detilleux, 2011) that considers a homogeneous population of size N in which a disease is spreading. Transmission of the disease occurs via direct animal–to–animal contact. Once infected, hosts are able to transmit the infection and are able to be re-infected. The infectious dose is assumed to depend on the pathogen burden in the infected host and the resistance of the susceptible one. After infection, pathogens multiply in the tissue environment and an innate immune response is mounted against them. In the absence of infection, immune effectors (called "response cells" throughout the manuscript) cycle throughout the body. During the early immune response, these response cells are recruited actively to the site of infection. Once they reach the site, they are activated and begin their task of digesting and destroying the invading pathogens.

For one individual, the within-host model follows the dynamics of response cells and pathogen populations:

$$\begin{aligned} B_{t+\Delta t} &= B_t + D_{t+\Delta t} + N_{t+\Delta t} - K_{t+\Delta t} \\ C_{t+\Delta t} &= C_t + M_{t+\Delta t} + G_{t+\Delta t} - S_{t+\Delta t} \end{aligned} \quad (1)$$

where B_t is the concentration of pathogens and C_t is the concentration of response cells at time t. Both infection and response to infection occur during consecutive small time intervals $(t + \Delta t)$. Within a time interval, the host is infected by $D_{t+\Delta t}$ new pathogens while pathogens present within the host multiply $(N_{t+\Delta t})$ and are killed by response cells $(K_{t+\Delta t})$. In the absence of infection, $M_{t+\Delta t}$ response cells reach the tissues while an extra-concentration $(G_{t+\Delta t})$ is recruited and removed $(S_{t+\Delta t})$ in case of infection. All concentrations are homogeneous Poisson processes: the number of events in time interval $(t + \Delta t)$ follows a Poisson distribution with associated specific rates that are described more specifically in the following section.

The symbol $D_{t+\Delta t}$ represents the concentration of pathogens effectively transmitted and inoculated to one host after contact with a number I of infective hosts, each infected with B_t^i pathogens. It is governed by the equation:

$$D_{t+\Delta t} = \sum_i c\nu\beta^i B_t^i \quad \text{for} \quad i = 1, 2, \cdots I,$$

where c = probability of contact between the host and an infective host, ν = the fraction of the infective dose actually inoculated by the host, and β^i = fraction of B_t^i the infected host excrete during an effective contact. Stated otherwise, $\beta\ B_t^i$ is the infective dose released by an infected host and ν represents the host anti-infection resistance.

The concentration of pathogens resulting from reproduction $(N_{t+\Delta t})$ is controlled by their multiplication rate, here assumed to be logistic:

$$N_{t+\Delta t} \sim \text{Poisson}[\gamma\ B_t(1 - B_t/K_B)]$$

In the equation, the per-capita growth rate (γ) is a function of the ability of pathogens to multiply until they reached their maximum concentration (K_B). This behavior has indeed been observed in well-mixed *in vitro* suspensions (Malka et al., 2010).

Concurrently to infection, response cells are activated to kill $K_{t+\Delta t}$ pathogens:

$$K_{t+\Delta t} \sim \text{Poisson}[\alpha C_t\ B_t\rho/(1 + (\tau\alpha B_t))]$$

where α is the maximum kill rate, τ is the time necessary for the cell to capture and kill the pathogen, and ρ is a scaling parameter representing the relative level of resistance of the host with theoretical limits at 0 or 1. If $\rho = 0$, the host is not resistant at all and cannot recover. The level of resistance is maximum at $\rho = 1$. In the science of ecology, the equation is called the Holling Type 2 functional response that describes the average feeding rate of a predator (here, a response cell) when the predator spends some time searching for a prey (here, a pathogen) and some time, exclusive of searching, processing each captured prey (Holling, 1959).

In the second part of equation [1], $M_{t+\Delta t}$ is the normal concentration of response cells in the tissue environment:

$$M_{t+\Delta t} \sim \text{Poisson}[\omega(C_1 - C_t)]$$

where ω is the natural rate at which cells are recruited and removed due to death or migration.

When pathogens are present, an extra-concentration of cells is recruited:

$$G_{t+\Delta t} \sim \text{Poisson}[\mu B_t C_t/(K_m + B_t)]$$

where μ is the maximum rate of recruitment and K_m is the half-saturation constant. Then, if B_t is low, $G_{t+\Delta t} \sim$ Poisson $[\mu C_t/K_m]$, and reaches $G_{t+\Delta t} \sim$ Poisson $[\mu C_t]$ when B_t is high.

The symbol $S_{t+\Delta t}$ represents the extra-removal of response cells after infection:

$$S_{t+\Delta t} \sim \text{Poisson}[\alpha C_t B_t \rho/(\theta(1+\tau\alpha B_t))]$$

The rate is called the numerical response rate (change in predator concentration as a function of change in prey concentration) and corresponds to the above Holling type II functional rate.

A response cell kills on average θ pathogenic cells before removal.

SYSTEM OF EQUATIONS FOR PERFORMANCE

Only the effects of pathogen and cells concentrations on hosts performances are considered. All other effects, such as resource intake, management or age are assumed fixed. Then,

$$P_t = P_1 - [B_t L_B(1-\lambda_b) + C_t L_c(1-\lambda_c)] \quad (2)$$

where P_t is the performance of the host in the presence of B_t pathogens and C_t response cells. The parameters L_B and L_C are the maximum performance lost per pathogen (virulence) and response cell, respectively. The parameters λ_b and λ_c are scaling parameters representing the relative ability of the host to tolerate damages caused by pathogens and immune cells. If $\lambda_b = \lambda_c = 1$, the host is completely tolerant and produces at the initial level ($t = 1$). If $\lambda_b = \lambda_c = 0$, the host is not tolerant at all. Although unrealistic (Can an animal be totally tolerant or un-tolerant?), the scaling parameters set the limits for P_t with a maximum at P_1 and a minimum at $P_1 - B_t L_B - C_t L_C$.

Resistance and tolerance are associated with a redistribution of resources away from performance:

$$P_1 = P^{\text{Max}}(1 - \rho c_p - \lambda_b c_b - \lambda_c c_c)$$

where P^{Max} is the maximal level of performance reached when levels of resistance and tolerance are null ($\rho = \lambda_b = \lambda_c = 0$). The parameter c_ρ is the relative costs of resistance while c_b and c_c are the relative costs of tolerance to pathogens (direct) and response cells (indirect).

VALUES FOR THE PARAMETERS

Values for parameters describing a healthy and early inflammatory response to infection are from studies on *E. coli* bovine mastitis (**Table 1**). Baseline values insure a healthy response such that pathogens are cleared and hosts return to pre-infection equilibrium. For the simulation, endemics start in a population of 50 susceptible hosts in which 2 are infected with concentrations of cells and bacteria close to 10^7cells/μL, and 10^6 bacteria/μL, respectively. The value 10^6 bacteria/μL is the highest concentration observed in neutropenic cows (Rainard and Riollet, 2003) and 10^7 cells/μL is the highest somatic cells concentration observed in a field survey of mastitis in Belgium (Detilleux et al., 2012). In non-infected hosts, concentrations of response cells (C_1) are normally distributed with mean of 100 cells/μL and standard deviation of five cells/μL (Djabri et al., 2002). Once inside the hosts, bacteria grow at a rate of one new pathogen per hour and response cells migrate to the site of infection with a maximum migration rate of 2 μL/bacteria/h (Detilleux et al., 2006). The time for a response cell to capture and kill the pathogen and the concentration of bacteria killed per cell were set at 1 h (Adinolfi and Bonventre, 1988) and 10 bacteria (Nagl et al., 2002), respectively. The Holling Type II kill rate is

Table 1 | Symbol, signification and values of the parameters.

Symbol	Signification	Values
PARAMETERS WITH THE SAME VALUES IN ALL SIMULATIONS		
K_B	Maximum concentration of pathogens	10^6/μL
K_C	Maximum concentration of response cells	10^7/μL
P^{Max}	Maximum performance	100 units
γ	Pathogen logistic growth rate	1 pathogen/μL/h
τ	Time for a response cell to capture and kill pathogens	1 h/cell
θ	Pathogen concentration killed per response cell	10 pathogens/cell
c	Contact rate between hosts	0.1/h
PARAMETERS FOR THE DIFFERENT RESPONSE SCENARIOS		
K_M	Pathogen concentration such that response cells reach the infection site in 1 time unit	
	Healthy response (scenario A)	10 cells/μL
	Recurrent infection (scenario B)	10000 cells/μL
α	Pathogen clearance rate	
	Healthy response (scenario A)	0.005 pathogen/cell/h
	Persistent infectious response (scenario C)	0 pathogen/cell/h
ω	Recruitment/elimination rate of response cells during health	
	Healthy response (scenario A)	0.5 cells/h
	Persistent non-infectious response (scenario D)	0.01 cells/h
μ	Extra-recruitment rate of response cells during infection	
	Healthy response (scenario A)	2 cells/μL/h
	Immuno-depression (scenario E)	0 cells/μL/h
PARAMETERS WITH UNIFORM DISTRIBUTIONS		
β	Infectiousness	U[0; 0.01]
L_C	Loss associated with each response cell	U[0; 25/K_C]
L_B	Loss associated with each pathogen	U[0; 25/K_B]
c_ρ, c_b, c_c	Resistance, direct and indirect tolerance costs	U[0; 0.1]
ν	Resistance to infection	
	Low	U[0; 0.001]
	Average	U[0; 0.01]
	High	U[0.009; 0.01]
ρ	Resistance to disease	
λ_b, λ_c	Direct and indirect tolerances	
	Low	U[0; 0.1]
	Average	U[0; 1]
	High	U[0.9; 1]

0.005/μL/bacteria/h (Detilleux et al., 2006). This means that as few as 0.005 bacteria are killed per cell and per h when B_t is small, and up to five bacteria are killed when B_t is high.

Outcome of the inflammatory response is not always health. To determine whether the model could reflect such reality, scenarios for the inflammatory response, other than the healthy response (scenario A), were tested by modifying the values of the parameters (Kumar et al., 2004). In scenario B, response cells are not recruited rapidly to the site of infection, pathogens cannot be completely eliminated and the infection is recurrent. In scenario C, infection is persistent and infectious when response cells and pathogens concentrations are high; it is persistent and non-infectious when pathogens are cleared but response cells concentrations are high (scenario D). The last scenario (scenario E), severe immunodeficiency, occurs when pathogens multiplied up to saturation with no activation of response cells.

Without information in the literature, values for the rates in equations for performance were drawn from uniform distributions. A convenient value of 100 units was given to P^{Max}. Individual levels in resistance and tolerance were drawn from distributions with different extreme values to have low (U[0, 0.1]), average (U[0, 1]), or high (U[0.9, 1]) levels. The maximum part of P_1 available to resistance and tolerance was set at $P^{\text{Max}}/2$. Individual tolerance and resistance costs were drawn from U[0, 0.1]. Highest direct (L_B) and indirect (L_C) loss associated with each pathogen were set at 25×10^{-6} units of performance lost per pathogen present, and 25×10^{-7} units of performance lost per response cell. The values for L_B and L_C were chosen to insure that P_t remains positive when costs and cell and pathogen concentrations are highest.

COMPUTATIONS

All computations were done on SAS 9.1. Simulation steps were executed until t reaches 100 time-units or the disease dies out (= one cycle) and repeated over 1000 cycles. At the end of all cycles, individual performance (P_t) and concentrations of host cells (C_t) and pathogens (B_t) were expressed as the percentages of their maxima (P^{Max}, K_C, and K_B, respectively), averaged over all animals and all replications, and plotted across time. Similarly, the number of infected hosts (I_t) was expressed as the percentage of the total number of hosts in the population (50) and averaged over all replications. To sum up, area under the curves of P_t (AUC_P) and I_t (AUC_I) were computed for $t = 1$–100 with the trapezoidal rule. Least-squares means of the AUCs were computed for high, average and low levels of tolerance and resistance.

RESULTS

This section starts with results about the ability of the model to simulate different scenarios of response to infection, at the animal

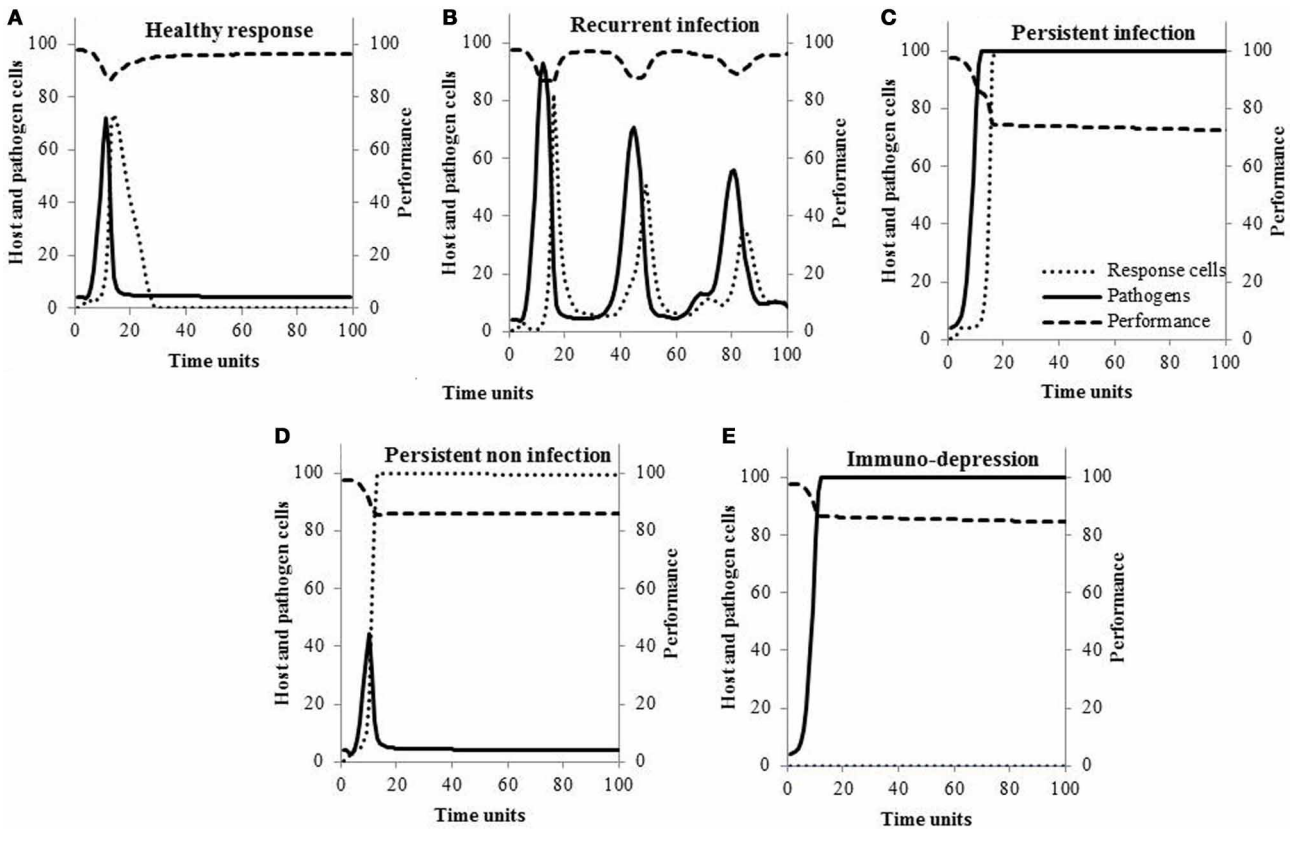

FIGURE 1 | Typical changes in the within-host concentrations of pathogens, response cells, and hosts performance according to the scenario of response to infection.

and population levels. It follows by the effects of different levels of resistance and tolerance on a healthy response.

SCENARIOS OF RESPONSE TO INFECTION AT THE ANIMAL LEVEL

Typical within-host curves are shown in **Figure 1** for the five different scenarios of response to infection. The concentrations of pathogens increase within 20 time units and are followed by an increase in response cells. If the response is healthy (scenario A), pathogens are killed efficiently by the response cells, their concentrations decrease, and host performance returns to pre-infection values. This is contrary to what is observed when pathogens susceptibility to the response cells is null (**Figure 1C**): concentration of response cells reaches high values but pathogens cannot be cleared (scenario C). In **Figure 1B**, the increase in response cells is delayed so pathogens are not completely eliminated. Then, the infection is recurrent and associated with episodes of performance losses (scenario B). If the response is persistent and non-infectious (scenario D), the concentration of response cells remains elevated even though pathogens are killed. In the last scenario (**Figure 1E**), response cells are not activated and pathogens grow to saturation (scenario E).

SCENARIOS OF RESPONSE TO INFECTION AT THE POPULATION LEVEL

Figure 2 shows how the infection spreads in the population. If hosts are able to get rid of the infection (**Figures 2A,D**), the endemics dies out. If the infection is recurring within the host, so do the endemics at the population level (**Figure 2B**). In case of persistent infectious response and immune-depression (**Figures 2C,E**), hosts all become infected.

RESISTANCE AND TOLERANCE ON WITHIN-HOST DYNAMICS

In **Figure 3**, concentrations of pathogens (B_t) during an episode of infection are shown for different levels of resistance to infection, concentrations of response cells (C_t) are shown for different levels of resistance to disease, and host performances (P_t) are shown for different levels of direct and indirect tolerance, all for hosts with a healthy response to infection. Peak of pathogen concentrations are high when both levels of resistance are low (**Figures 3A,B**). Similarly, concentrations of response cells necessary to fight pathogens increase when cell levels of resistance to disease and to infection move from high to low (**Figures 3C,D**). Performances decreased during the response to infection (**Figure 3E**) unless the host is highly tolerant to damage associated with both pathogens and response cells (line "High-High"). When both direct and indirect tolerance levels are lowest (line "Low-Low"), the loss during the period of infection is the highest with P_t going from 98% at $t = 0$ to 86% at $t = 13$ time-units but the loss is the lowest over the period from $t = 0$ to $t = 100$ time-units. Indeed, the loss from $t = 0$ to $t = 100$ time-units varies from 4.5% of the maximum performance (P_{Max}) if the host is not tolerant (line "Low-Low") to 10.9% if it is highly tolerant (line "High-High"). It is 7.2% and 8.3% if the host is tolerant to direct (line "High-Low") or indirect (line "Low-High") damages, respectively.

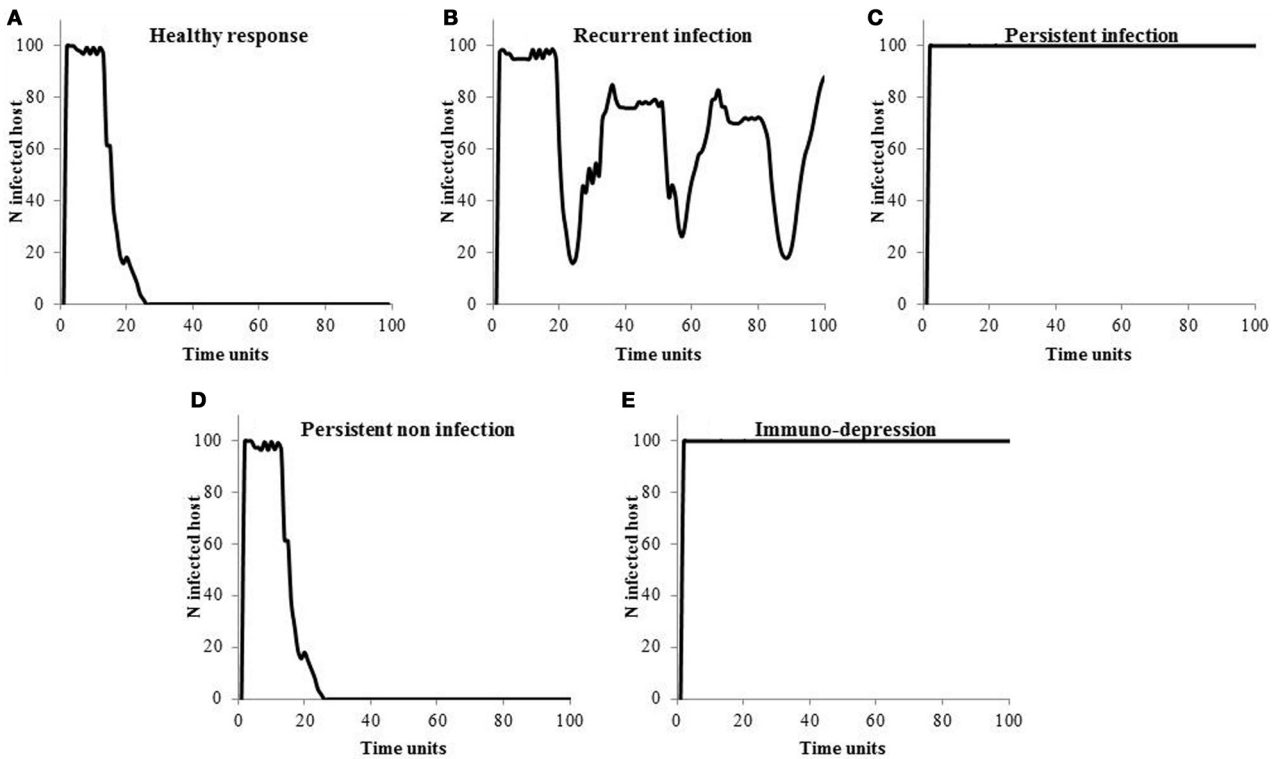

FIGURE 2 | Size of the endemics according to the scenario of response to infection.

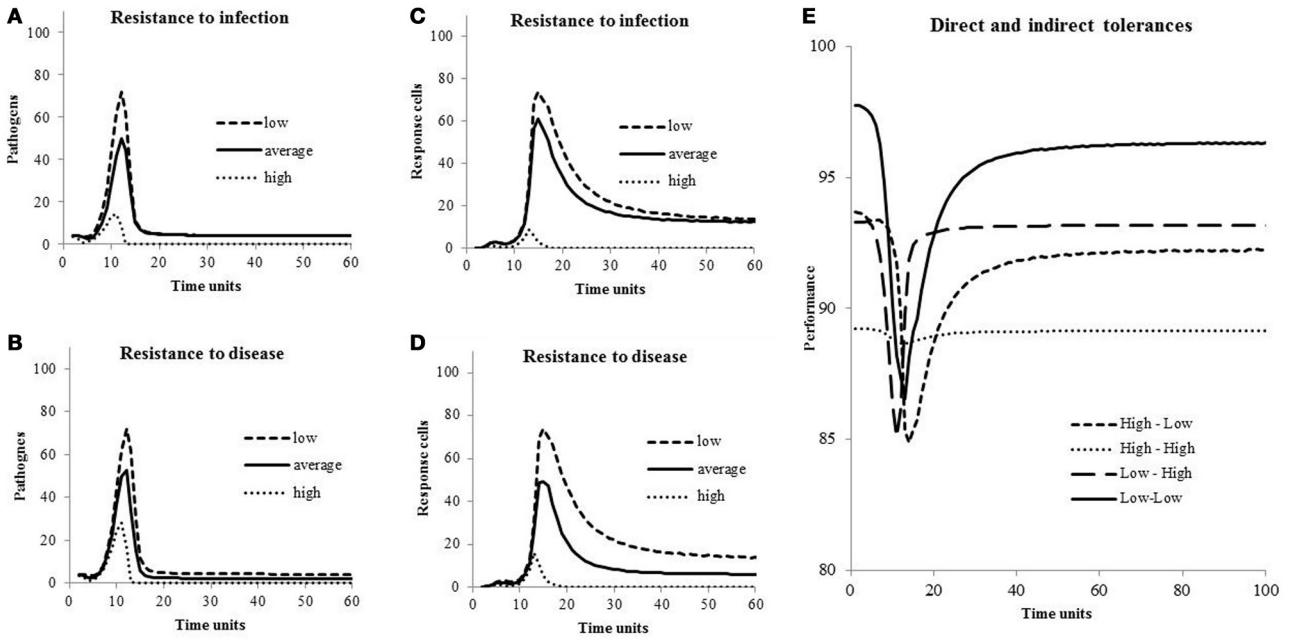

FIGURE 3 | Concentrations of pathogens and response cells for different levels of resistance to infection and to disease, and host performances for different levels of direct and indirect tolerances. Values of the parameters are in **Table 1**.

RESISTANCE AND TOLERANCE AT POPULATION LEVEL

The area under the curves of performances (AUC_P) and number of infected hosts (AUC_I) are shown in **Figure 4** for high, average and low levels of resistance to infection and disease, and for high, average and low levels of direct and indirect tolerance. The AUC_P is the highest (most favourable) when hosts mount a healthy response to infection, are highly resistant to disease and infection, and not tolerant to both direct and indirect damages associated with the infection. It is the lowest when hosts are persistently infected, not resistant to disease and infection, and highly tolerant to both direct and indirect damages associated with the infection. The AUC_I is the highest (most favourable) when hosts are immunodepressed or persistently infected with low levels of resistance to infection and disease. It is the lowest when hosts mount a healthy or persistent response to infection and are highly resistant to infection and disease. Indirect and direct levels of tolerance have no effect on AUC_I.

DISCUSSION

A mathematical model is proposed to quantify the effects of resistance and tolerance on the spread of an infectious disease (here, *E. coli* mastitis) and on animal performances within a closed population. Such theoretical studies are necessary because resistance and tolerance are difficult to be measured in field studies. Actually, resistance is typically assessed by measuring infection intensity, i.e., bacteriological cultures in the case of bovine mastitis. However, such information is often lacking because it is time-consuming and costly to obtain. Indirect measures of infection intensities (e.g., somatic cell counts, conductibility, and clinical signs) have also been proposed but their accuracy in evaluating the udder bacteriological status is low. Even when the information is available, different intensities may be the fact of different levels of resistance but also of different chances of encountering pathogens. Indeed, a susceptible animal in a population free from the pathogen has no opportunity to get infected and may be erroneously classified as resistant. When infection intensity is available, one can also measure tolerance as the slope of a regression of host performance against infection intensity. But this measure does not distinguish between direct and indirect tolerances. Costs of resistance and tolerance are even more difficult to quantify in practice since their measures necessitate evaluating hosts performances in pathogen-free environments (Råberg et al., 2009).

If they are necessary, models should also adequately reflect reality. Although simple, the model proposed here allows simulating scenarios that have all been observed in animals. For example, changes in pathogens and cell concentrations depicted in **Figure 1A** (scenario A) were previously described in cows experimentally infected with different *E. coli* doses (Vangroenweghe et al., 2004). Burvenich et al. (2003) showed phagocytes with low killing ability (**Figure 1C**) cannot sustain an effective elimination of the pathogen and the resolution of *E. coli* mastitis (scenario C). It is also known that cows suffering from the leukocyte adhesion deficiency syndrome (scenario E) present persistent infection (**Figure 1E**) due to the lack of molecules necessary for neutrophils to migrate out of the blood stream toward the site of infection (van Garderen et al., 1994). As a final example, Hill (1981) showed infections can persist and lead to recurrent clinical mastitis (**Figure 1B**) when the speed at which neutrophils are mobilized in the gland is low (scenario B).

Within the range of selected values (**Table 1**), the model suggests breeding should be for animals mounting a healthy

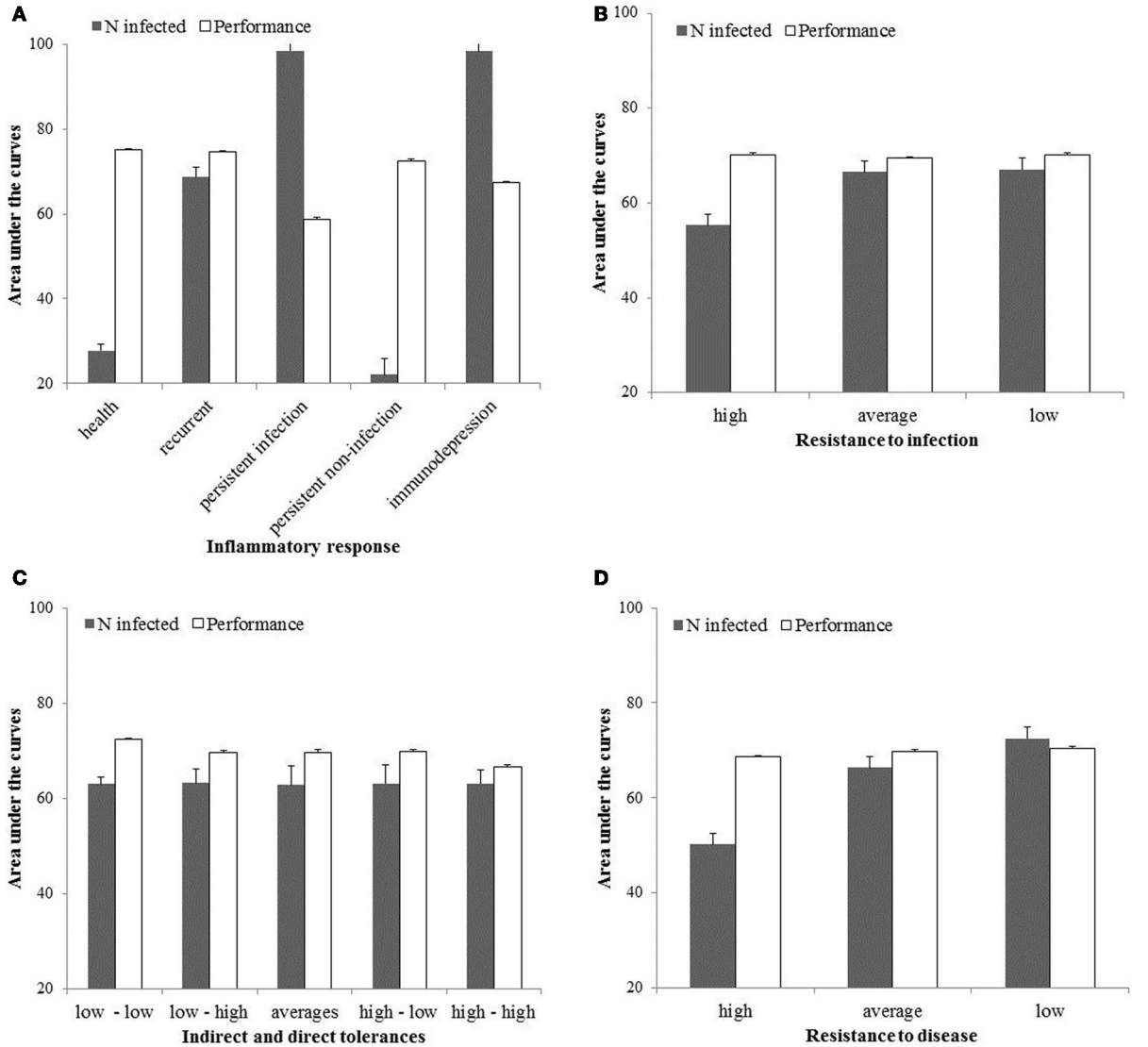

FIGURE 4 | Area under the curves for the host performances and number of infected in the population for the first 100 time-units and expressed as the percentages of their respective maxima.

response to infection and highly resistant to disease or infection. Then, performances at the population level will be the highest and endemics the smallest (**Figure 4**). In this particular situation and if resistance is independent of tolerance, improving tolerance should not be considered as a selection objective because it is redundant to resistance: an already resistant host will not get infected or diseased so energy is not necessary to tolerate damages linked with infection. Note however several mechanisms have been shown to influence both resistance and tolerance (Shinzawa et al., 2009; Ayres and Schneider, 2012) so selection for resistance can result in a correlated response in tolerance. Other potential factors that may influence the decision of whether improvement of resistance is beneficial over improvement of tolerance have been ignored in this model. These may include host-pathogen co-evolution (e.g., Roy and Kirchner, 2000), infection-induced reduction in resource intake (e.g., Sandberg et al., 2006) or different shapes of cost functions associated with resistance and tolerance (e.g., Restif and Koella, 2004).

Values for costs and effects of resistance/tolerance on host performances were chosen arbitrarily because no information was found in the literature. An exception is the experiment of Råberg et al. (2007, 2009) on laboratory mice inoculated with the rodent malaria parasite *P. Chabaudi*. They observed approximately 10% decrease in weight and red blood cell density per $3\,\mu L^{-1} \times 10^6$ parasites. But even though they are based upon arbitrary values, effects shown in **Figure 3** are relatively coherent: Pathogen and response cells concentrations increase when resistance to both infection and disease decreases (**Figures 3A–D**). During an episode of infection, losses in performance are highest in hosts

not tolerant to damage associated with the presence of pathogens and the response to infection (**Figure 3E**, line "Low-Low"). But, on a period of $t = 1$–100 time-units, the loss is the smallest because not tolerant hosts have set little resources away from performances and return to higher performance levels after the episode of infection. Note this loss is around 5% of the maximum performance and corresponds, luckily, to the loss in milk production associated with clinical mastitis case at the lactation level (meta-analysis of Seegers et al., 2003).

Another drawback of the model is that hosts in the population all present one particular scenario of response to infection (**Figure 2**) although studies suggest a genetic influence on the response to infection (Davies et al., 2009). For example, in Holsteins, heritabilities have been reported for neutrophils migration (0.2–0.5), for neutrophils phagocytosis (0.3–0.7), for cellular-mediated adaptive response (0.16) and for antibody-mediated adaptive response (0.2–0.4) (Detilleux et al., 1994; Thompson-Crispi et al., 2012). To account for these differences, the model could easily be made more realistic by simulating different scenarios for different individuals and also for different periods in the same individual.

No link was considered between scenario of response to infection and costs of resistance/tolerance, considering that costs were constitutive and allocated in a pathogen-free environment (Rohr et al., 2010). But one may argue that animals with more resources for defense-related pathways, rather than performance, will preferentially mount a healthy rather than another type of response. They may defend themselves by over-expressing specific defense pathways in a temporal and spatial manner rather than wide-ranging constitutive mechanisms (Medzhitov et al., 2012). Conversely, too many resources could lead to excessive immune response and immuno-pathology (Colditz, 2002). So, the question remains whether selection objective should be for animals with constitutive or inducible resistance/tolerance.

In conclusion, the model is useful in shedding some light on the complex interactions between resistance/tolerance and performance but needs realistic values to better grasp the processes. To improve it, we are planning a small explorative study, funded by the European research group EADGENE_S, to measure animal levels of resistance/tolerance to bovine mastitis in herds located in Wallonia. Resistance will be measured by the number of bacteria colony forming units in milk of cows located in herds in which cows' opportunity to get infected is measureable (Detilleux et al., 2012). Direct and indirect tolerances will be quantified with structural equation models linking somatic cell counts, colony forming units and milk production. Hopefully, this will give us some clues on how to choose our selection objectives and improve the health of animals in an economic environment.

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REFERENCES

Adinolfi, L., and Bonventre, P. (1988). Enhanced phagocytosis, killing, and serum sensitivity of *Escherichia coli* and *Staphylococcus aureus* treated with Sub-MICs of Imipenem. *Antimicrob. Agents Chemother.* 32, 1012–1018.

Ayres, J., and Schneider, D. (2012). Tolerance of infections. *Annu. Rev. Immunol.* 30, 271–294.

Bonnefont, C., Rainard, P., Cunha, P., Gilbert, F., Toufeer, M., Aurel, M., Rupp, R., and Foucras, G. (2012). Genetic susceptibility to *S. aureus* mastitis in sheep: differential expression of mammary epithelial cells in response to live bacteria or supernatant. *Physiol. Genomics* 44, 403–416.

Boots, M., and Bowers, R. (1999). Three mechanisms of host resistance to microparasites; avoidance, recovery and tolerance, show different evolutionary dynamics. *J. Theor. Biol.* 201, 13–23.

Burvenich, C., Van Merris, V., Mehrzad, J., Diez-Fraile, A., and Duchateau, L. (2003). Severity of *E. coli* mastitis is mainly determined by cow factors. *Vet. Res.* 34, 521–564.

Canale, C., and Henry, P.-Y. (2010). Energetic costs of the immune response and torpor use in a primate. *Funct. Ecol.* 25, 557–565.

Colditz, I. (2002). Effects of the immune system on metabolism: implications for production and disease resistance in livestock. *Liv. Prod. Sci.* 75, 257–268.

Davies, G., Genini, S., Bishop, S., and Guiffra, E. (2009). An assessment of opportunities to dissect host genetic variation in resistance to infectious diseases in livestock. *Animal* 3, 415–436.

Detilleux, J. (2011). Effectiveness analysis of resistance and tolerance to infection. *Genet. Sel. Evol.* 43, 9.

Detilleux, J., Koehler, K., Freeman, A., Kehrli, M., and Kelley, D. (1994). Immunological parameters of periparturient Holstein cattle: genetic variation. *J. Dairy Sci.* 77, 2640–2650.

Detilleux, J., Theron, L., Beduin, J.-M., and Hanzen, C. (2012). A structural equation model to evaluate direct and indirect factors associated with a latent measure of mastitis in Belgian dairy herds. *Prev. Vet. Med.* [Epub ahead of print].

Detilleux, J., Vangroenweghe, F., and Burvenich, C. (2006). Mathematical model of the acute inflammatory response to *Escherichia coli* in intramammary challenge. *J. Dairy Sci.* 89, 3455–3465.

Djabri, B., Bareille, N., Beaudeau, F., and Seegers, H. (2002). Quarter milk somatic cell count in infected dairy cows: a meta-analysis. *Vet. Res.* 33, 335–357.

Eadgene. (2012). *Success Stories*. EU Contract No. FOOD-CT-2004–506416.

FAO. (ed.). (2007). "Section E: animal genetic resources and resistance to disease," in *The State of the World's Animal Genetic Resources for Food and Agriculture*, (Rome: FAO), 101–111.

Hill, A. (1981). Factors influencing the outcome of *Escherichia coli* mastitis in the dairy cow. *Res. Vet. Sci.* 31, 107–112.

Holling, C. (1959). Some characteristics of simple types of predation and parasitism. *Can. Entomol.* 91, 385–398.

Kover, P., and Schaal, B. (2002). Genetic variation for disease resistance and tolerance among *Arabidopsis thaliana* accessions. *PNAS* 99, 11270–11274.

Kumar, R., Clermont, G., Vodovotz, Y., and Chow, C. (2004). The dynamics of acute inflammation. *J. Theor. Biol.* 21, 145–155.

Malka, R., Shochat, E., and Rom-Kedar, V. (2010). Bistability and bacterial infections. *PLoS ONE* 5:e10010. doi: 10.1371/journal.pone.0010010

Medzhitov, R. (2009). Damage control in host–pathogen interactions. *PNAS* 106, 15525–15526.

Medzhitov, R., Schneider, D., and Soares, M. (2012). Disease tolerance as a defense strategy. *Science* 335, 936–941.

Nagl, M., Kacani, L., Mullauer, B., Lemberger, E.-M., Stoiber, H., Sprinzl, G., Schennach, H., and Dierich, M. (2002). Phagocytosis and killing of bacteria by professional phagocytes and dendritic cells. *Clin. Diagn. Lab. Immunol.* 9, 1165–1168.

Nunez-Farfan, J., Fornoni, J., and Valverde, P. (2007). The evolution of resistance and tolerance to herbivores. *Annu. Rev. Ecol. Evol. Syst.* 38, 541–566.

Oltenacu, P., and Algers, B. (2005). Selection for increased production and the welfare of dairy cows: are new breeding goals needed? *Ambio* 5, 311–315.

Råberg, L., Graham, A., and Read, A. (2009). Decomposing health: tolerance and resistance to parasites in animals. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 364, 37–49.

Råberg, L., Sim, D., and Read, A. (2007). Disentangling genetic variation for resistance and tolerance to infectious diseases in animals. *Science* 318, 812–814.

Rainard, P., and Riollet, C. (2003). Mobilization of neutrophils and defense of the bovine mammary gland. *Reprod. Nutr. Dev*. 43, 439–457.

Restif, O., and Koella, J. C. (2004). Concurrent evolution of resistance and tolerance to pathogens. *Am. Nat.* 164, E90–E102.

Rohr, J., Raffel, T., and Hall, C. (2010). Developmental variation in resistance and tolerance in a multi-host–parasite system. *Funct. Ecol.* 24, 1110–1121.

Roy, B. A., and Kirchner, J. W. (2000). Evolutionary dynamics of parasite resistance and tolerance. *Evolution* 54, 51–63.

Sandberg, F. B., Emmans, G. C., and Kyriazakis, I. (2006). A model for predicting feed intake of growing animals during exposure to pathogens. *J. Anim. Sci.* 84, 1552–1566.

Sears, B., Jason, R., Allen, J., and Martin, L. (2011). The economy of inflammation: when is less more? *Trends Parasitol.* 27, 382–387.

Seegers, H., Fourrichon, C., and Beaudeau, F. (2003). Production effects related to mastitis and mastitis economics in dairy cattle herds. *Vet. Res.* 3, 475–491.

Shinzawa, N., Nelson, B., Aonuma, H., Okado, K., Fukumato, S., Miura, M., and Kanuka, H. (2009). p38 MAPK-dependent phagocytic encapsulation confers infection tolerance in *Drosophila*. *Cell Host Microbe* 6, 244–252.

Thompson-Crispi, K., Sewalem, A., Miglior, F., and Mallard, B. (2012). Genetic parameters of adaptive immune response traits in Canadian Holsteins. *J. Dairy Sci.* 95, 401–409.

van Garderen, E., Muller, K., Wentink, G., and van den Ingh, T. (1994). Post-mortem findings in calves suffering from bovine leukocyte adhesion deficiency (BLAD). *Vet. Q.* 16, 24–26.

Vangroenweghe, F., Rainard, P., Paape, M., Duchateau, L., and Burvenich, C. (2004). Increase of *Escherichia coli* inoculum doses induces faster innate immune response in primiparous cows. *J. Dairy Sci.* 87, 4132–4144.

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