

The emergence and evolution of swine viral diseases: to what extent have husbandry systems and global trade contributed to their distribution and diversity?

T.W. Drew

Virology Department, Veterinary Laboratories Agency (Weybridge), Woodham Lane, New Haw, Addlestone, Surrey KT15 3NB, United Kingdom

Summary

Over the last 20 years, pig production has been characterised by a rapid increase in the volume of pig meat produced, greater intensification of the pig-rearing process and much greater international movement of products. There have also been many novel viral diseases that challenge the industry. Are these two developments linked and, if so, how?

To understand how changes in the industry may influence the evolution of viruses, it is important to understand something of evolutionary theory. For RNA viruses, the concept of 'quasispecies' has moved solidly from theory to fact. Such viruses do not exist as a single entity, but as a 'cloud' of viruses, whose degree of diversity is influenced by a number of factors. Chief among these are the size and rate of the replicating population, along with the availability and diversity of susceptible hosts. A feature of RNA viruses is a high level of mutation, due to lack of capability to correct errors on the part of the host cell. Both *in vivo* and *in vitro*, RNA viruses have been shown to accumulate and fix these mutations, leading to bottleneck events and fitness loss, the phenomenon known as 'Muller's ratchet'.

Likewise, the opposite effect, fitness gain, can be achieved in an environment providing for high levels of replication and the generation of large populations of virus. This has been shown to be possible *in vitro* by high-volume passage. It is possible that the regular introduction of diverse viruses within large-scale pig production provides an *in vivo* equivalent that could drive quasispecies populations to increased fitness, and may explain why emergent viruses, either new to science or with new synergies and presentation, seem to be appearing more commonly.

Keywords

Emerging viruses – Foot and mouth disease virus – Husbandry – International trade – Novel viruses – Pigs – Porcine circovirus-2 – Porcine reproductive and respiratory syndrome virus – Quasispecies – Swine influenza virus – Virus evolution.

Introduction

Pork production has increased markedly over the last few decades, emphasising its importance as a global protein source. This pressure for increased efficiency and a greater

volume of production has led to increased intensification of production, along with more frequent movements of pigs, germplasm and pig meat throughout the last two decades.

This trade movement has primarily been from more competitive countries to those with less efficient

production systems, which has led to a domination of the market by efficient countries and the gradual disappearance of inefficient production systems in the latter. The impact of this decline can be significant, both economically and sociologically, since the role of small-scale pig production in some countries is interwoven into society; as well as food, village pigs represent a dowry, a savings bank and a source of fuel. Furthermore, small-scale production is often part of a mixed-farming operation, which utilises pigs to consume crop by-products or excess grain. In contrast, large-scale production invariably involves units dedicated to pig production, sometimes alongside a separate industry producing custom feed; although this is sometimes also commercially integrated into the pig production system itself. The industry today is increasingly dominated by large international corporations, operating as individual or multi-site integrated units, sometimes with separate ownership, operating units and geographical locations for each phase of production. In these integrated systems, movements of pigs and germplasm also play an important part.

The viral diseases encountered today within the pig industry have never been more diverse. Some diseases, such as classical swine fever, are still a major problem in some areas of the world and a constant threat to others. But there are also viruses, newly emerged, that have surprised and confounded us. Some have been contained, others have become established as pathogens of major economic impact and importance. In some cases, their origins are known whilst, for others, the mechanism of their emergence is not. But, in all cases, international trade seems to have played a significant role in their distribution and, in some instances, has also contributed to their evolution, in various ways.

This paper examines the basis of viral evolution itself and discusses the emergence of a number of key viral pathogens of pigs, and how their evolution has been facilitated by the move to large-scale agriculture and associated trade in pigs and pig products.

Global and regional pig production figures

Globally, pork is the most popular meat. Furthermore, worldwide consumption of pork and pork products increased by 27% from 1997 to 2005, with total global pork consumption for 2005 standing at more than 93 million tonnes.

Since 2008, world pig meat production has increased by about 2% per year, and is currently at 106.5 million tonnes (23). There has been a significant increase in production in

the People's Republic of China (hereafter referred to as China), which now accounts for half of world pig meat production, but also in some other countries, such as Canada, Mexico and Vietnam, where pork accounts for nearly three-quarters of the total meat production (1). In contrast, there has been some contraction in pork production in the European Union, the United States and the Republic of Korea, where high feed costs and concerns over the effects of influenza type A/H1N1 have led to reduced consumer demand for pig meat.

Despite the upturn in production, prices have fallen. *Agricultural Outlook 2010-2019*, jointly published by the Organisation for Economic Co-operation and Development (OECD) and Food and Agriculture Organization of the United Nations (FAO) (38), shows pork to have been the only one of 17 commodities which decreased in value from 2007 to 2008, and the ten-year forecast is similarly gloomy. This will undoubtedly increase pressure on producers to reduce production costs as much as possible. It remains unclear how this will be achieved, but it is likely that increased industrialisation in developing countries will feature.

The germplasm trade

The international exchanges of animal genetic resources from 1990 to 2005 were recently analysed by Gollin *et al.* (25), who noted that 95% of the flow was from Europe and the United States to developing countries, with a dramatic increase seen between 2000 and 2005. The effect of this flow of genetic resources is the displacement of the indigenous animal genetic resources of developing countries, along with a resultant increasing level of genetic homogeneity among global pig populations. The impact of this is hard to judge, but it has been suggested that this trend to a porcine 'monoculture' is to the detriment of natural resistance to pathogens and may even provide opportunities for the more rapid spread of newly emerging pathogens.

The key drivers of viral evolution

Before discussing some key pig viral diseases, it is appropriate to review briefly the concept of viral diversity and evolution. Obviously, the attributes of a virus are encoded within its genome. What is less obvious is that the genome of a virus is never fixed. Any virus population comprises a mixed population of nucleotide sequences. In DNA viruses, this population is rather limited, because of the cellular proof-reading mechanisms that exist for

DNA replication, which also ensure high fidelity during DNA virus replication. However, in RNA viruses, proof-reading does not occur, so there is a much higher mutation rate. As a consequence, RNA viruses exist as a 'cloud' of variants, consisting of the total number of variations that can exist for any given viral genome. This 'quasispecies' concept (18, 19) is not just a mathematical theory, it has a real basis in the biology of viruses and, in particular, of RNA viruses (11, 13). Although quasispecies theory has been around for many years, its relevance to viral evolution is generally poorly understood.

Within a viraemic animal infected with an RNA virus, there exist populations of viruses or a 'mutant spectrum', generated by the replication process and whose genetic makeup is therefore subtly variable. Of course, there are a large number of constraints placed on this variation. Many variants with changes in critical areas will be non-viable, by virtue of changes that render key elements of the infection process defective. However, there is a selection process at work, which can tolerate certain changes, provided other changes also occur. And, in some cases, there might be a change which is potentially advantageous to the replication and dissemination of a particular virus clade within a mutant spectrum, either in the current environment of the virus, or in a new environment. In this way, the evolutionary process is constantly at work. For viruses that have been present in pigs for many years, the relationship tends to be more fixed, resulting in the emergence of one or more stable ensembles of the genome; more often referred to as genotypes or serotypes. Even so, each ensemble will itself consist of a variant mutant spectrum, with the viral sequence of a particular isolate defining only the major population within the mutant spectrum present at that time within the sample obtained from a particular animal.

These RNA viral populations will constantly be subjected to changes in the ways that pigs are raised, thus providing new environments that select new populations of virus variants. Pig immunity can also be considered an environmental factor, which can itself be affected by a number of other factors, including genetics, age and maternal factors, nutrition, pollutants, vaccination regime, stress and the presence of further viral, bacterial or other pathogens. All these factors can affect the ability of a virus to cause disease, and also ultimately influence the makeup of the progeny viral population that may emerge from the infected animal.

The relative ability of a particular virus population to replicate under specified environmental conditions is defined as its 'fitness' (10, 15). This is a complex biological parameter, which varies, depending on the amount of virus that is present. This is because the two primary factors, random shift (the replication errors and recombination) and positive selection (the ability of all members of the

diverse population to reach a new host cell), are both influenced by the size of the viral population and the availability of susceptible hosts. Transmission of large populations often results in fitness gain, whilst repeated bottleneck events lead to average fitness losses (9, 28).

This can be demonstrated in the laboratory, using two simple but very different methods of producing virus stocks *in vitro*, essentially measuring difference in fitness gain. Serial passage in cell culture, by repeatedly taking supernatants from one culture onto a fresh one, results in profound fitness gain (12), whilst repeat plaque purification, taking a virus population from a single virus plaque, results in an accumulation and fixation of genetic mutations, producing the bottleneck effects of 'Muller's ratchet' (the phenomenon whereby once the least mutated genomes in an asexual population begin to carry at least one deleterious mutation, no genomes with fewer such mutations can be expected to be found in future generations) and fitness loss (7, 29) (see Fig. 1). This fitness loss is also seen *in vivo* during serial passage in animals (5).

One can, therefore, postulate that the environment provided by high-density pig production would strongly

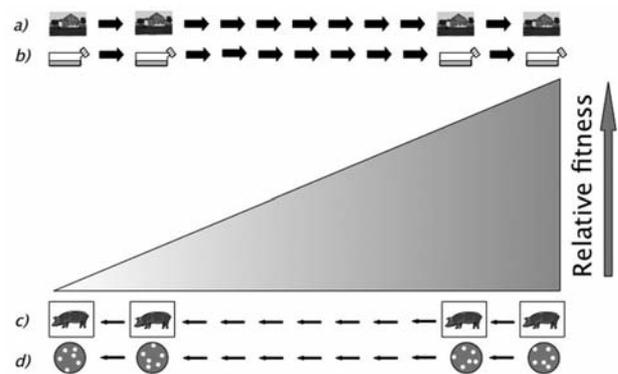


Fig. 1
A simplified schematic of fitness variation of a virus following replication in a constant environment

A hypothesis is presented that *in vivo* passage in two different farming systems imposes the same opportunities for fitness gain and loss as their *in vitro* counterparts. Large population passages (a = large pig farms with pigs of homogeneous genetics and age and b = sequential passage in monolayer cell culture) result in fitness gain. In these circumstances, random shift is minimal and mutant viruses with higher replication efficiency will predominate. Passages involving very small amounts of virus, and/or with sequential bottleneck events (c = transmission between small isolated village pig populations and d = plaque picking) tend to result in fitness loss. Under these conditions, random drift will tend to prevail and genomes with higher replication efficiency will have little chance of becoming dominant in the quasispecies population. The population size needed to initiate a changed level of fitness within a given system will depend on the initial fitness of the virus population for the environment. Adapted from (12)

favour fitness gain, in the same way as a cell monoculture in the laboratory, and may lead to increased replicative and infectious efficiency on the part of viruses in such a system, possibly with higher pathogenicity, since susceptible hosts are plentiful and rapid death is unlikely to compromise transmission. In contrast, village pig production may result in fitness loss, manifest as lower virulence viruses, with lower viraemias, due to a series of bottleneck events, as has been described for many viruses following serial passage in individual or very small numbers of animals.

The influence of 'mutant spectra' in virus evolution

The evolutionary advantages for a virus population to exist as a spectrum of subtly different genomes is intuitively obvious; namely, that there is, encoded within the variants, the opportunity to benefit from a mutation, either in the existing environment or in a changed one. Domingo *et al.* (13) have defined five parameters that determine the complexity of the mutant spectrum: average number of mutations per genome, virus population size, genome length, relative fitness and mutations needed for a phenotypic change. Mutations that may be phenotypically relevant (i.e. change some viral characteristic) have the potential to strongly influence the development of new mutant distributions in an environment that favours them. This may be the existing environment, or a new one.

However, mutant spectra can also exert a modulating effect in some circumstances. Even if a particular mutant virus has the capacity to become dominant within a quasispecies, it may not necessarily do so. It all depends on the mutant spectra that surround that mutant. If an inferior mutant is surrounded by a large number of closely related mutants, in contrast to a fitter mutant that is either unique or with low numbers of related mutants, the inferior mutant will dominate (19). This is a difficult concept to understand intuitively but can be proven mathematically, and has also been observed *in vivo*. Probably the most cited example is that of polio vaccine, where it was discovered that only when a minority virulent poliovirus was above a certain defined concentration in a preparation could it overcome the attenuated poliovirus vaccine strain (of inferior relative fitness) and cause neurological disease in vaccine recipients (8).

This interplay among the mutant spectra within the quasispecies population results in a constant flux, with changes in the environment, such as introduction to new host populations, providing an additional variable that may lead to the emergence of new dominant spectra. This is the backdrop behind the theatre of disease emergence or re-emergence and why viral diseases seem to gain or lose importance over time.

Viral diseases of key importance to the swine industry today

Disease can affect pig production in two main ways; through piglet mortality and poor weight gain. Of course, more pathogenic diseases, such as classical swine fever, will kill pigs of all ages, but this is the exception rather than the norm. In endemic areas, vaccination is extensively used, and outbreaks in disease-free areas are rare. It is generally only when new diseases emerge, or known diseases without a vaccine are introduced to new areas, that significant mortality is seen.

There have been some spectacular examples of emerging diseases in pigs over the last 20 years. Nipah virus is a good example. The disease first appeared on mainland Malaysia in 1998 and caused heavy losses among pigs in the region and also, disturbingly, infected humans, causing over 200 human deaths. Yet, despite the serious nature of this emergence, its overall impact on pig production has been minimal, apart from placing restrictions on the siting of pig farms in the region, where avoidance of bat colonies is a crucial component of control. Other viruses, such as Menangle and Bungowannah, have also recently been discovered in pigs in Australia, with the former being linked to bats. The source of the latter is as yet unknown. Yet other viral diseases have not only recently emerged or re-emerged, but have had a much greater impact, largely because efforts to control them have failed, often due to the novelty of the agents themselves. Some examples are reviewed below.

Porcine reproductive and respiratory syndrome virus

There is probably little doubt that, for the pig industry, the single most important recently emerging disease is porcine reproductive and respiratory syndrome (PRRS). The disease was first described in Canada in 1987, also spreading rapidly south into the United States. The first outbreak in Europe was in Germany, in 1990, and scientists at Lelystad, the Netherlands, were the first to describe the causative virus, PRRS virus (PRRSV). It soon became apparent that the causative viruses in the two continents were different, though clearly of the same family. The viruses were originally termed 'European' and 'North American', but these geographical labels are no longer appropriate, given the international spread of the disease, and they are now termed type 1 and type 2 respectively.

Within a very short time, the disease spread to Asia, probably via live pigs or semen, where it rapidly became

established in pig populations throughout the region. A particular feature of PRRSV is its ability to mutate and evolve rapidly. Initially, viruses within each of the two populations were relatively similar, though the type 2 strains were more diverse. But, within two or three years, similar levels of diversity were seen with type 1 viruses and, today, consequent with more extensive analysis of Eastern European strains, the diversity of type 1 strains is considered equal to or greater than type 2. Indeed, there is even emerging evidence of a distinct clade in Eastern Europe which some might regard as a putative 'type 3' PRRSV.

However, the evolution of type 2 viruses continues, with significant diversity evolving in China (49). The most significant event to have occurred with this virus is the emergence of a highly pathogenic variant of a type 2 virus in that country, which has now spread throughout Southeast Asia, and also to the Philippines and Indonesia. A molecular marker, a deletion in non-structural protein 2, characterises this variant (43), though it is unclear whether this deletion is directly related to virulence (21).

So why does PRRSV exhibit such a propensity for rapid evolution? There are certainly two main attributes that facilitate this. First, it is an RNA virus, with a consequent innate error-prone replicative process which itself generates quasispecies. Secondly, as with all members of the *Coronaviridae*, the molecular mechanism of its replication involves a three-step process from a full-length, positive strand RNA to a full-length negative strand, from which are generated a number of subgenomic messenger RNAs. The possibility for further evolution is provided by the arrangement of the open reading frames (ORFs) within the genome of the virus. By utilising all three frames within the triplet code, portions of the ORFs overlap each other, so that two amino acids, in two different proteins, may be encoded by a single nucleotide sequence. The triplet nucleotide code contains an element of redundancy, with many amino acids coded by more than one nucleotide triplet, often the third nucleotide, often termed a 'wobble-base'. However, for PRRSV, if a single nucleotide transcriptional error occurs in the overlapping region, there is a greater chance of at least one amino acid change and the error may induce two changes.

However, these attributes alone do not account for the dramatic evolution exhibited by this virus since its first appearance. Other arteriviruses, such as equine arteritis virus and lactate hydrogenase-elevating virus of mice, do not exhibit such evolution or diversity. A likely explanation, at least in part, is that this is a novel virus to pigs and is still adapting. The density of pigs in Asia has possibly provided the opportunity for numerous large population passages, thus increasing the relative fitness of PRRSV for its host. The relative novelty of this virus for the pig host and the environment in Asia have led to the

emergence of a more virulent form, also seeming to infect older pigs, which now seems to predominate and displace other strains of the virus in the region.

Foot and mouth disease

Although foot and mouth disease virus (FMDV) could not be regarded as novel, an outbreak in 1997 among pigs in Chinese Taipei (6, 41) had some particular features which made it so. The outbreak was a devastating blow to the farmers and veterinary authorities of that country, resulting in approximately 38% of pigs in Chinese Taipei dying or being slaughtered. Pig mortality and speed of spread were high, with nearly a million pigs on infected premises alone, and the cost of the outbreak was estimated to have been in excess of US\$378 million. What is interesting, from an evolutionary perspective, is that the virus involved in the outbreak, designated FMDV O/Tw/97, was found to have a strong adaptation for pigs. Early in the outbreak, it was noted that the disease seemed to be restricted to pigs, with no clinical signs reported in ruminants, even though there was clear evidence of contact. The porciphilic nature of this isolate and its apparent non-infectivity for cattle was also demonstrated experimentally at the World Reference Laboratory for FMD in Pirbright, the United Kingdom (17). Phylogenetic analysis indicated that the virus was related to type O viruses from Hong Kong and the Philippines (44). Although no direct epidemiological links with mainland China were identified, it was concluded that the most likely source of the outbreak was pigs smuggled into Chinese Taipei from the mainland.

How did this virus become adapted to pigs; are there any genetic markers that correlate with this apparent stabilisation in the pig and just how stable is it? Foot and mouth disease virus infects a wide range of species, with productive infection and clinical signs in most cloven-hoofed animals. Early literature describes naturally occurring variations in disease signs, attributed to viral variation, such as the ability to cause myocarditis, neuropathology or diabetes (for a review, see 14). The known determinants of virulence and host range for FMDV include a capsid 'RGD' receptor motif, which binds to a number of cellular integrins and is known to be involved in cellular tropism in natural infections. This motif is required for an infection to produce clinical signs (26, 32). However, *in vitro*, other non-integrin-dependent cell binding can be demonstrated, either by multiple *in vitro* passages, by induction of escape mutants or by molecular engineering (2, 36, 48). Such mutants invariably demonstrate reduced virulence or its absence, and this approach has been proposed as being of value in the development of live vaccines. Studies of the basis of the host restriction of FMDV O/Tw/97 revealed that a deletion in the non-structural protein 3A was involved (3, 27), with evidence of the accumulation of mutations in viruses from

the region having built up over the previous 30 years or so (27). These workers also showed that substitution for this deletion, using an infectious clone, resulted in loss of the porcine host restriction. Carrillo *et al.* (5) carried out an interesting experiment, by serially passaging an isolate of FMDV O/Tw/97 20 times in pigs, transmitting the virus each time solely by contact. This yielded a rather unexpected result; namely, that there was a steady reduction in the virulence of the virus, culminating in no clinical disease by passage 14, but with virus present in nasal swabs and the tonsil in pigs up to the end of the experiment, at passage 20. The authors observed that this result mimicked the fitness loss induced by plaque-to-plaque FMDV passage *in vitro* (the 'bottleneck effect') and suggested that unknown mechanisms of virulence recovery might be necessary during the evolution and perpetuation of FMDV in nature. Another explanation is the hypothesis presented here; not that it is an unknown mechanism of virulence, but simply that large collections of susceptible animals in close proximity provide an environment very unlike that of serial passage. In large herds, high levels of virus production, efficient transmission and multiple exposures may combine to produce a population of sufficient size and fitness to drive the relative fitness of the quasispecies population circulating within the herd in the other direction – towards fitness gain.

Swine influenza

Swine influenza is a serious disease, most often manifesting as seasonal respiratory disease among young pigs. But the disease can be endemically and continuously present at low prevalence, with less obvious disease, such as reproductive effects in sows and boars.

The emergence of a new pandemic influenza A virus in humans in March 2009 (34), in which evolution in pigs was seen as a significant factor, caused much global concern. However, emergence of a new strain of influenza A in pigs is not an unusual event but occurs regularly, due to the nature of the pathogen and the pig host. Influenza A viruses are inherently unstable because they are RNA viruses, they can infect many different species and have segmented genomes. This provides opportunities for significant change within the genome of the virus, leading to the generation of extremely diverse quasispecies populations under certain circumstances.

The epidemiology and evolution of influenza A viruses are complex and a number of comprehensive general reviews are available (30, 31, 35, 39, 45).

Influenza A viruses are classified by their haemagglutinin (H) and neuraminidase (N) subtypes. Additionally, sequence analysis can indicate the origin of different segments of the genome, based on earlier analyses from

pigs and other host species. Currently, three genotypes of influenza virus predominate among pig populations worldwide: H1N1, H3N2 and H1N2. Within these major groups are 'classical' swine H1N1 (previously only found in North America), avian-like H1N1, human-like H3N2, reassortant H3N2 and a number of different H1N2 viruses. European strains of these subtypes differ from those found in North America and Asia and various lineages exist within each subtype. In Asia, a classical swine H1N1 and human-like H3N2-type virus predominate. The differences between the swine influenza viruses on these continents indicate that there has been little mixing of the virus populations, but with the introduction of swine influenza viruses to Asia at some time in the past.

Birds are the main host for influenza A, as evidenced by the fact that all known subtypes infect avian species. Viruses found circulating in avian species preferentially bind to sialic acid 2,3 galactose receptors, in contrast to those infecting humans, where only subtypes H1, H2 and H3 are found regularly. This is because, in humans, a different receptor, sialic acid 2,6 galactose, is thought to be preferentially employed.

Pigs are known to have both receptors and have, therefore, been traditionally regarded as 'mixing vessels', with potential for the generation of new reassortant viruses following co-infection. But this does not explain why the general restriction of pigs and humans is the same. In theory, avian viruses should be able to infect pigs via the 2,3 receptor. However, recent studies of receptor distribution (34) have shown that the distribution in pigs is little different from that in humans, with significant differences being observed in the intestinal tract only. These studies concluded that pigs are no more likely to be potential hosts for virus re-assortment than humans. However, the recent emergence and spread of the human A H1N1 virus has been shown to have evolved over some years in pigs, before its emergence as a human pathogen (42). There is also subsequent evidence of its re-introduction to pigs and further evolution (46), demonstrating the dynamic nature of the evolution of this virus in pigs.

Porcine circovirus-2

Other emerging viral diseases have been more insidious in their arrival and impact. An example is porcine circovirus type 2 (PCV-2). So subtle was the arrival of this pathogen that it still remains a subject of debate as to whether it is causative or synergistic. What is certainly true is that this virus can precipitate significant disease, in which other known primary or opportunistic pathogens are also involved. Today, this group of diseases is generally known as PCV-associated disease (4). But field disease has been rather difficult to reproduce experimentally. However,

indirect, indisputable evidence of the significance of its involvement in disease is provided by the dramatic improvement in piglet health since the launch of killed PCV-2 vaccines.

As a DNA virus, PCV-2 is considered very stable, so it is difficult to explain its emergence as a pathogen without also considering host genetics and other factors, such as husbandry and the possibility of immuno-compromisation through vaccination of the young piglet against other diseases.

The evolution of circoviruses generally is an interesting subject, since, as DNA viruses, they are inherently less prone to mutation and are also very small, presumably with less redundancy. A recent analysis of the evolution of PCV-2 (37) revealed that PCV-2 could be phylogenetically divided into two subgroups (1 and 2), with eight clusters (1A to 1C and 2A to 2E). However, no specific association with pathogenicity was found within these subgroups. Likewise, neither was there any association with geography, although this is not surprising, given the nature of the industry and the length of time that PCVs have been present in pigs. The analysis of Olvera *et al.* (37) did, however, reveal possible evidence of recombination within cluster 1B. Also, evidence of selective pressure was detected in all parts of the PCV-2 genome, especially in the *rep* gene, indicating that host-driven evolution has occurred.

Perhaps the most interesting recent development in the circovirus story is the emergence of a chimeric PCV-1/PCV-2 in Canada (4), associated with clinical disease, in which PRRSV was also implicated. It is tempting to hypothesise that the likely source was from the incomplete inactivation of a chimeric virus in an inactivated vaccine used in the region. However, there is no evidence for this and Gagnon *et al.* (24) raise the possibility that the genomic material in the vaccine might have acted as a DNA vaccine and resulted in productive virus, citing the work of Fenaux *et al.* (22) as a demonstration of this phenomenon, for PCV-1, PCV-2 and a chimeric clone. If this hypothesis is proven, it would certainly raise concerns about the use of inactivated, engineered vaccines, whose safety is solely dependent on that inactivation and which have, therefore, generally been regarded as not posing any risk.

The insidious nature of the disease and initial poor understanding of the role of PCV-2 has meant that, early in its emergence, formal controls were lacking. As a consequence, there has been a high likelihood of introduction to new areas (16).

The importance of other viruses, such as torque teno virus (20, 40) and bocavirus (47), which are similar in nature to circoviruses, is currently unclear. But if such involvement

is proven, it is likely that they will also become subject to testing in the context of international trade.

Conclusions

The World Organisation for Animal Health publishes recommendations for safe international trade in animals and their products in the *Terrestrial Animal Health Code* (*Terrestrial Code*). These recommendations have contributed significantly to reducing the incidence of disease outbreaks through the unwitting carriage of disease from one country to another. For their part, national veterinary authorities should also regulate movements and ensure that testing is performed to the standards required. It remains to be seen whether the *Terrestrial Code* can keep up with, and control the spread of, emerging diseases.

It has not been the purpose of this article to review the numerous examples of disease introduction where legal or illegal trans-national transport of animals or derived material was the source. Rather, the article has aimed to provide sufficient evidence to show that the environment provided to viruses by the global intensification of pig farming and associated international movements has contributed to their ever-increasing diversity. Evolutionary benefits accrue to RNA viruses due to the nature of their replication, in particular, which can lead to improved relative fitness in the environment provided by the high-density agriculture increasingly practised in many countries. Current solutions to the challenge of virus emergence and re-emergence focus primarily on developing prophylactic vaccines to reduce the susceptibility of pigs, along with barriers at farm and international level to reduce the risk of spread. But we know that prevention is impossible. We can, at best, only reduce the risk.

Perhaps a better approach would be to understand more of the molecular basis of viral evolution and develop methods to intervene. Evolutionary biomathematicians have begun to examine if it is possible to impair viral replication by intervening in the interactions of mutant spectra, thus inducing information 'meltdown' or 'error catastrophe'. That is, essentially driving the relative fitness to zero. If this could be achieved, it offers the exciting prospect of a whole new field of vaccine design, which targets the replicating virus directly, rather than via the immune system of the pig.

However, until such theories become reality, the dangers inherent in large-scale pig production and international trade in pigs, pig meat and germplasm must be recognised. We must understand that the high density and almost clonal nature of pig genetics can provide a 'monoculture' environment which may select highly pathogenic viral

clades, leading to explosive outbreaks of novel disease. This drives an ever-increasing demand for new vaccines, which, in this age of molecular engineering, may themselves potentially pose a threat. Also, this dual 'pathogen and prophylactic' challenge to the young piglet can be formidable and it is debatable whether it can be sustained. It is the nature of evolution that the fittest within a given environment proliferate, so it is logical that, if such proliferation is encountered, it is the environment that is the driver. The industry, the scientists who serve it and the veterinary authorities that regulate it all need to re-evaluate the key drivers behind the many novel disease problems being encountered in pig production and the soundness of the current scientific approaches being applied to solve them.

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Émergence et évolution des maladies virales porcines : jusqu'à quel point les systèmes d'élevage et le commerce international contribuent-ils à la diversité et à la distribution de ces maladies ?

T.W. Drew

Résumé

Depuis une vingtaine d'années, la filière porcine connaît une hausse rapide des volumes de viande produits, ainsi qu'une intensification croissante des systèmes d'élevage et une multiplication des transferts internationaux de produits dérivés du porc. Dans la même période, la filière porcine a également été touchée par l'apparition de nombreuses maladies virales nouvelles. Ces deux évolutions ont-elles un lien entre elles, et si oui, lequel ?

Pour bien appréhender l'influence que peuvent avoir les changements industriels sur l'évolution des virus, il est impératif d'avoir des connaissances de base sur la théorie de l'évolution. S'agissant des virus à ARN, le concept de « quasi-espèce » n'est plus seulement théorique mais désigne désormais une réalité concrète. Ces virus ne constituent pas un groupe caractéristique, mais plutôt un « nuage » de virus plus ou moins différenciés en fonction de plusieurs facteurs. Parmi ces facteurs, les plus déterminants sont la taille et la capacité répliquative des populations virales, ainsi que la disponibilité et la diversité des hôtes sensibles. L'une des caractéristiques des virus à ARN est leur importante capacité de mutation, due à l'absence de mécanisme correcteur de la part des cellules hôtes. *In vivo* comme *in vitro*, les virus à ARN sont capables d'accumuler et de fixer ces mutations, ce qui conduit à des goulets d'étranglement répétés et à une perte de la capacité répliquative, phénomène connu sous le nom de « cliquet de Muller ».

De manière symétrique, lorsque le contexte favorise des niveaux élevés de répliquative et la production de grandes populations virales, l'effet inverse se produit, à savoir une amélioration de la capacité répliquative des virus. C'est ce qui se produit *in vitro* lors de passages dans des volumes importants. On peut donc envisager qu'une introduction régulière de différents virus dans un système de production porcine à grande échelle fournisse *in vivo* des conditions équivalentes et favorise l'amélioration de la capacité répliquative des populations de quasi-espèces virales, ce qui pourrait expliquer l'apparition apparemment

plus fréquente de virus émergents, jusque-là inconnus des scientifiques ou dotés de synergies et de propriétés inédites.

Mots-clés

Circovirus-2 porcin – Commerce international – Élevage – Évolution des virus – Porc – Quasi-espèce – Virus de la fièvre aphteuse – Virus de la grippe porcine – Virus du syndrome dysgénésique et respiratoire du porc – Virus émergent – Virus nouveau.



Aparición y evolución de enfermedades porcinas víricas. ¿Hasta qué punto han contribuido a su distribución y diversidad los sistemas de producción animal y el comercio internacional?

T.W. Drew

Resumen

En los últimos veinte años, la producción porcina se ha caracterizado por un rápido incremento del volumen de carne producida, una mayor intensificación del proceso de cría y un movimiento internacional de productos mucho más nutrido. También han aparecido un gran número de nuevas enfermedades víricas que plantean dificultades a la industria. ¿Están vinculados entre sí todos esos hechos? Y en tal caso, ¿de qué manera?

Para comprender la forma en que la transformación de la industria puede influir en la evolución de los virus, es importante entender ciertos aspectos de la teoría de la evolución. En el caso de los virus de ARN, el concepto de 'cuasiespecie' ha pasado del terreno teórico al de los hechos tangibles. Tales virus no existen como fracciones únicas, sino como 'nubes' de virus cuyo grado de diversidad depende de múltiples factores, de los que los más importantes son el tamaño de la población replicante y su tasa de replicación, junto con la disponibilidad y diversidad de hospedadores sensibles. Un rasgo de los virus de ARN es su elevado índice de mutaciones, que obedece a su incapacidad de corregir errores por parte de la célula hospedadora. Tanto *in vivo* como *in vitro* está demostrado que los virus de ARN acumulan y fijan esas mutaciones, lo que conduce a situaciones de cuello de botella y pérdida de aptitud (en el sentido evolutivo del término), fenómeno que se conoce como el 'trinquete de Muller'.

Análogamente, en un medio que propicie elevados niveles de replicación y generación de poblaciones víricas de gran tamaño puede producirse el efecto opuesto, esto es, la mejora de la aptitud. Se ha demostrado que ello es posible *in vitro* mediante pases en volumen. Tal vez la introducción repetida de diversos virus en explotaciones de producción porcina industrial ofrezca condiciones equivalentes *in vivo*, capaces de llevar a poblaciones de cuasiespecies a adquirir mayor aptitud evolutiva, lo cual puede explicar por qué parece que estén apareciendo con más frecuencia virus emergentes, ya sean éstos nuevos para la ciencia o virus conocidos que se acompañan de nuevas sinergias o formas de presentación.

Palabras clave

Cerdos – Circovirus porcino 2 – Comercio internacional – Cuasiespecie – Evolución de los virus – Nuevos virus – Producción animal – Virus emergentes – Virus de la fiebre aftosa – Virus de la gripe porcina – Virus del síndrome disgenésico y respiratorio porcino.



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