Visions on the future of veterinary virology

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Introduction

One of the most common perceptions of a virus is to look at it as an enemy, a pathogen, a "disease-causing germ". In veterinary virology, this usually translates into "XY virus causes a devastating (severe, economically important) disease in this or that animal species and may (even) be transmitted to man". Indeed, many viruses have been recognized as the causative agents of diseases, such as Rinderpest, Foot-and-Mouth Disease, Malignant Catarrhal Fever, African and Classical Swine Fever, Pseudorabies, Transmisssible Gastroenteritis, Canine Distemper, Feline Parvovirosis, Rabies, and Influenza - to name but a few. On the other hand, viruses with little or no known pathogenicity did not stir much interest, were neglected at best, if not ridiculed in the scientific community. Examples include bovine herpesvirus type 4, canine parvovirus 1, porcine circovirus, toroviruses, and lentiviruses in general, at least before the advent of the AIDS epidemic.

Indeed, research was focused mainly on the disease-causing viruses. Adaptation of viruses to grow in cell culture and the increasing knowledge of molecular biology have contributed most to the expanding field of virology in the second half of the 20th century.

As illustrated in Fig. 1, the number of appearances of the keyword "virus" in Medline from 1960 to 1999 reveals two stepwise increases, the first one in the mid-sixties reflecting the progress made in molecular biology or, more specifically, in genetic engineering. The second step in the mid-seventies coincides with the Asilomar conference, where scientists declared their willingness to carry the responsibility for their work, particularly in the field of genetic engineering. With the advent of the AIDS epidemic, an exponential increase of virus-related literature can be observed, which levelled into a plateau by the mid-nineties. In 1999 the keyword "virus" was found 11.686 times, not even reaching the number of quotes in 1994 (11.788 times). Is this statistic heralding the doom of virology?

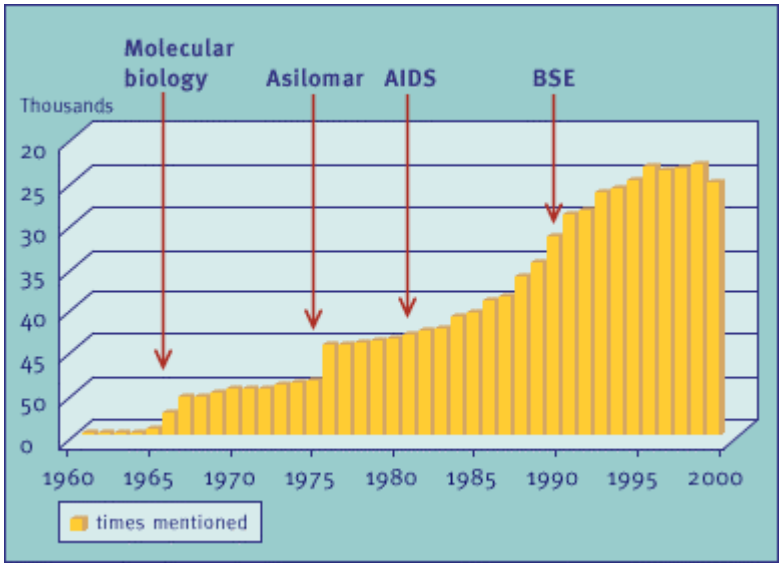

Fig. 1 Frequencies at which the keyword "virus" appeared in Medline (between 1966 and April 2000).

Emerging viruses and virus-like agents

The emergence of new viruses is not uncommon, as Table 1 shows. New viruses can be assigned to two categories at least: (i) newly evolved ones, like the influenza virus H5N1 Hong Kong [22] or the porcine respiratory coronavirus (PRCV) [14, 19], and (ii) newly discovered ones, like the Australian bat lyssavirus [10], the porcine circoviruses type 1 [28, 29] and type 2 [2], or Nipahvirus [5]. It may remain obscure into which category a particular virus fits, and one may safely assume that there are many viruses out there, which have not (yet) been recognized as disease agents.

New	Since
Infectious Bovine Rhinotracheitis virus	1953
Porcine circovirus type 1	1974
Canine parvovirus 2	1976
Human immunodeficiency virus	1980
Porcine respiratory coronavirus	1985
Bovine spongiforme encephalopathy	1986
Rabbit hemorrhagic disease virus	1986
Porcine respiratory and reproductive syndrome virus	1990
4-Corner Hantavirus	1993
Hendravirus	1994
Australian bat lyssavirus	1996
Influenza virus H5N1 Hong Kong	1997
Porcine circovirus type 2	1997
Nipahvirus	1999

Table 1 Overview of some new viruses and year of recognition.
So why should we perpetuate the dogma that viruses are always disease agents? On the other hand, should we really consider some viruses harmless, only because we have not (yet) seen their association with disease?

Co-evolution of viruses and host defence mechanisms

In our daily research, we use viruses to study the host's defence system and employ immunological tools to examine viruses. Despite of this mutual usefulness, the interaction between viruses and their hosts is most often pictured as a battle, a war-like situation. Therefore, only a total victory, the complete destruction of the enemy, i.e. total clearance of the virus from the organism, the population, the world, is considered as a basis for lasting peace. In veterinary virology, this view is reflected in political strategies to protect farm animals from highly contagious disease agents, such as those recorded in List A of the Office International des Épizooties (O.I.E.). Once the label "DANGEROUS!" has been attached to a particular virus, it can be removed only with great difficulty, mostly only after a change in the political climate. This fact leads to strange situations, as e.g. in the pestivirus field, where classical swine fever virus (CSFV) is in List A of the O.I.E. As a consequence, CSFV-infected herds must be destroyed and control measures must be taken to prove that the virus has been eliminated from the farm, the region, the country in question. In contrast, the closely related bovine virus diarrhoea virus (BVDV) and border disease virus - also pestiviruses - are considered as innocuous and consequently neglected by the veterinary authorities. This example emphasizes the most important argument, the seeming truism that neither the virus nor its host are static entities. Who can tell whether the vacuum created by the extinction of CSFV will not be filled soon by another virus?

The first encounter between a new or exotic virus and a particular host may result in a fatal outcome for both. This situation is illustrated by reports of encounters between seemingly new viruses and unprepared hosts, recently [5,18] and in the past (e.g. [23, 24]). The host's inadequate defence and/or the virus' poor adaptation may result in death

of the infected organism, and the virus would soon become extinct if it destroyed the susceptible population too rapidly. Hence, to survive in nature, any virus needs the host's functional defence as much as the host itself - both the host and the virus are on a path of co-evolution (Fig. 2) (e.g. [12], and references therein). Again: both are essential, the defence mechanisms for the survival of host and virus, and the virus for the constant education and evolution of the defence mechanisms.

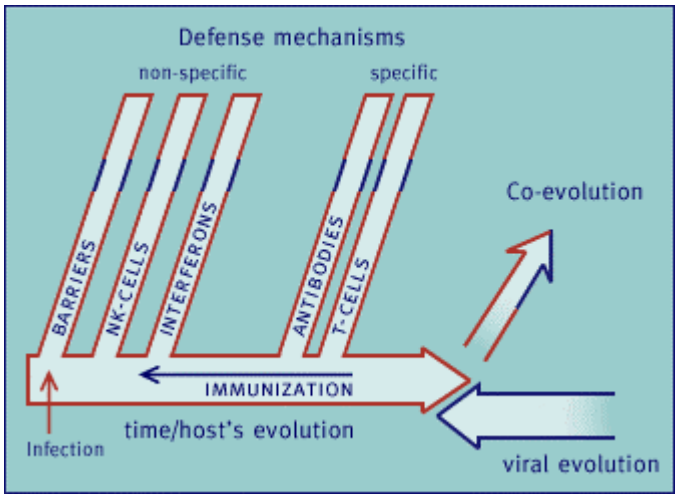

Fig. 2 Co-evolution of virus and the host's defence mechanisms

A dramatic example of this co-evolution, the HIV/AIDS epidemic, is occurring before our eyes. In Botswana, a shocking 35.8% of adults are infected with HIV (http://www.unaids.org/fact_sheets/files/Africa_Eng.html), which means that Africa will probably be repopulated by progeny of HIV-resistant survivors of the infection. Genetic determinants for long-term survival of AIDS have indeed been described [4,31], as have viral determinants, which influence the same [1].

In my opinion, the eradication of a given virus will create an empty ecological niche, soon to be taken by another agent, probably by one less adapted to the host than the original virus. Thus, if extermination of viruses is not the ultimate goal of veterinary virology, what is its goal? Obviously, the direction of co-evolution is influenced by external factors; understanding these factors is essential for steering the co-evolution of host and virus a course that is beneficial for humans and animals.

Development of vaccines

Modified live (mlv) and inactivated viral vaccines have a long history. In recent years, however, it has become customary to start developing and producing vaccines as soon as a new virus has been identified and adapted to growth in cell culture. In my view, this has resulted in a number of useless, if not dangerous vaccine preparations. One remarkable example is the field application of a modified live African Swine Fever (ASF) virus vaccine in Spain and Portugal, which almost led to the perpetuation of ASF on the Iberian peninsula ([12], and references therein).

Another example is the case of a canine coronavirus (CCV) vaccine. Although no disease could be associated with CCV infection [26], much effort was invested into developing a vaccine. To demonstrate its efficacy, immunized and control dogs were treated with dexamethasone after challenge, which resulted in diarrhoea in most (80%) control animals, as well as in some (15%) vaccinees [9]. Using this approach it was shown that dogs could be protected from a disease that does not even exist in nature.

The influenza viruses - much underestimated in veterinary virology - may serve as a third example. Between 1985 and 1991, several subtype H1N1 influenza A viruses had been isolated from patients in Mongolia. Characterization by sequence analysis [3] showed that these isolates were most closely related to strains isolated from diseased camels in the same region at the same time. The camel viruses were apparently derived from a UV-inactivated reassortant vaccine (PR8/USSR/77) that had been prepared for humans (in Leningrad, 1978) and used in the Mongolian population. The evidence was convincing: a reassortant between two human influenza virus strains had caused severe epizootics in camels, a species previously unknown to be a natural host for influenza A viruses.

Which lessons should we take home from these incidents? I suggest the following: (i) there is a need for better vaccines (Tab. 2) (ii) vaccine development should take the natural co-evolution of viruses and their hosts into account (iii) results obtained in model studies should be interpreted with caution (iv) it is very clear that mistakes have occurred in the past and more are bound to happen in the future. However, these mistakes should not discourage us. Vaccine development should continue with prudence and forethought, keeping the Japanese proverb in mind: "Vision without action is a daydream. Action without vision is a nightmare".

	'modified live'	ideal	inactivated
replication	yes	(yes)	no
attenuation	yes	yes/no	no
inactivation	no	(yes)	yes
stimulation	Th_1	Th_1 & Th_2	Th_2
(bio)risk	concern	no concern	little concern
basic immunization	once	once	multiple

Table 2 Properties of classic vaccines and the ideal preparation

Indeed, progress has recently been achieved in developing novel vaccination strategies, epitomized by DNA or RNA vaccines (reviewed in [15]) and by the BAC-VAC principle [25]. BAC-VAC refers to an artificial bacterial chromosome harbouring the entire genome of herpes simplex virus type 1 (fHSVΔpac), with minor deletions. The most important deletion concerns the signals for packaging of the viral DNA into virions. This bacterially cloned viral DNA is infectious and leads to replication, protein synthesis and virus particle formation upon gene gun application. However, the progeny is not infectious, because the viral DNA cannot be packaged. This single-cycle replication was found to induce cytotoxic T-cells (CTL), antibodies, and protection in a manner almost indistinguishable from immune reactions after mlv immunization - and it proved superior to inactivated or conventional DNA vaccines. Specifically, 1.5 μg of fHSVΔpac coated to gold particles yielded the same amount of CTLs as 10^9 $TCID_{50}$ of DISC HSV-1 [17], but five times higher antibody titers and antibodies of all isotypes; gB-specific CTLs could even be measured without previous restimulation in vitro. BAC-VAC immunized mice were protected against an intracerebral (i.c.) challenge infection with 200 LD_{50} of HSV-1, which leads to disease and death in control animals. Protection was cell-mediated, since serum transfer had no effect.

Apart from its potential to induce an immunity similar to that after infection, BAC-VAC is intrinsically safe: if ever the packaging signals would be restored by recombination during co-infection with a wild type virus, the immune system would profit from a lead over the multiplication of the recombinant, and development of disease or even spread of the vaccine virus would be quite unlikely.

Gene therapy

Casually, viruses have been paraphrased as "jumping genes" or "bad news in an envelope". Both references acknowledge their potential for gene therapeutic applications. From an opposite vantage point, a host species may profit from a virus as a population control agent, as a biological weapon that destroys competitors for limited natural resources by means of what we may flippantly call "contagious gene therapy"; these viruses may occasionally wreak havoc when a species is targeted, which did not undergo co-evolutionary adaptation (Table 3).

Virus	Aimed at	Secondary target
african swine fever virus	african bush pigs and warthogs	domestic pig
rinderpest virus	african free ranging ungulates	cattle
alcelaphine herpesvirus	wildebeest	cattle, elk
ovine herpesvirus 2	sheep	cattle
african horse sickness	unknown, zebra	horse
smallpox virus	old world man	new world natives
ebola virus	unknown	man

Table 3 Some viruses carry the potential of natural biological weapons.

However, a virus infection may also be looked at more favourably, as a special form of "gene therapy". The host acquires the genome of an infecting virus, and in the course of the ongoing virus-host interactions, a plethora of host genes is regulated. This occurs in a variety of manners, depending on the virus and the infected organism, and includes the multifaceted cascade of immune responses (see e.g. [11,30]. Ultimately, the infected organism will not only survive but also have acquired a selective advantage through an improved immune defence. In a long-term perspective, such virus-host interactions lead into the mentioned co-evolutionary pathway, with advantages on both sides. Thus viruses may also be viewed as movable genes or extrinsic genotypes of a host. At the same time, the virus profits from this mutuality by constantly probing for the opening of new ecological niches. In this case, the virus cannibalises genomic information from its host and modifies it in a manner that suits its replication and survival in nature.

From this perspective, the virus could be looked at as a companion, a helping hand, and it would seem plausible to ask one's friend for even more help. Recently, Luo and Salzman (2000) have compared the efficiency with the toxicity of DNA delivery systems [16]. They found that most often efficient delivery was obtained only at the expense of high toxicity (Fig. 3). The lowest relative toxicity associated with the highest efficiency was accredited to microinjection. Needless to say that viruses easily surpass the efficiency of manual microinjection.

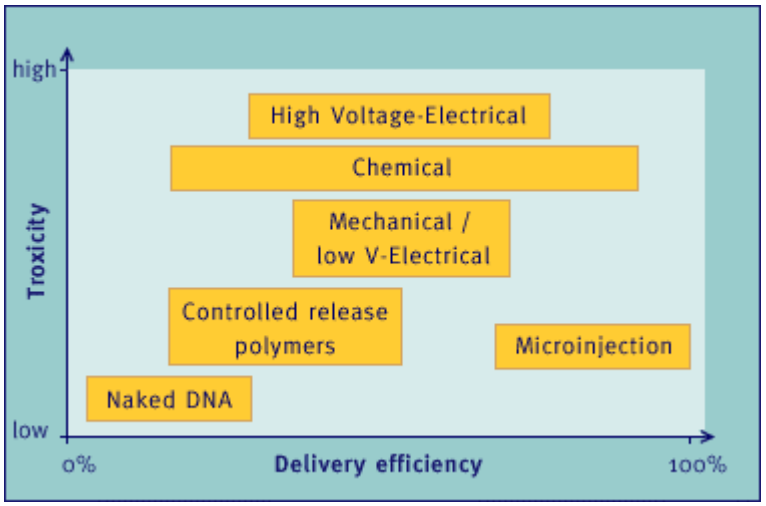

Fig 3. Efficiency versus toxicity of DNA delivery methods [16]

In Table 4 I have listed some advantages and disadvantages of viral vectors. In most cases advantages in one aspect are counterbalanced by disadvantages in other aspects.

Vector	Adeno	Herpes	Pox	AAV	AAV
Insert (kb)	8	>20	>25	>4.5	8
Titer (log)	11	8	>9	>9	6-7
Integration	no	no	no	yes	yes
Oncogenic	yes	?	?	?	yes
Viral proteins expressed	yes	yes	yes	yes	no
Expression	transient	poor	lytic	sustained	sustained
In vivo delivery	yes	yes	yes	yes	poor
Transmitted to quiescent cells	yes	yes	yes	yes	no

Table 4. Viral vectors [13]

Collaborative efforts between clinicians, gene therapy specialists and virologists have resulted in helpervirus-free vectors, which combine the advantages of different systems [6,20]. For example the large transgene capacity of up to 160 kbp and ability to target neuronal cells of herpes simplex virus amplicons was successfully combined with elements of the adeno-associated virus (AAV), which stands for sustained gene expression and the possibility to integrate the transgene at a specific location of the human chromosome 19 [7]. Moreover, a herpes simplex virus-Epstein-Barr virus hybrid amplicon was used to convert cells to producers of retrovirus vectors, which were enveloped for either ectotropic or amphotropic cell targeting in gene therapeutic applications [21].

These vectors are not only tools for the treatment of humans suffering from certain genetic diseases. They may be important for the analysis and modification of virus-host interactions in general. Thus herpetic stromal keratitis (HSK) is considered as virus-induced but sustained by the immune system [27]. The ongoing ocular inflammation can be suppressed by the topical administration of IL-10 DNA [8]. This report indicated that ongoing immunopathological events can be influenced by local gene therapy. There is an enormous potential for helpervirus-free amplicon vectors to modulating immunopathological diseases. By using them for gene delivery, most caveats associated with conventional viral vectors, as listed in Table 4, would appear manageable.

This may seem a truism, but I still want to emphasise: an enormous amount of virological knowledge will have to be collected before such visionary concepts will find their way into medical or veterinary applications.

Conclusions

Viruses are not necessarily disease agents; on the other hand, viruses currently not associated with disease may not be that harmless. Viruses and their hosts co-evolve, which has lead to more successful viruses in the face of improved host defence mechanisms. New and better vaccines are needed but should not be developed without taking the co-evolution of viruses and their hosts into account. The use of viruses as vectors for gene therapy is not without risks - after all, they are disease agents. However, if treated with respect and studied with care and foresight, they carry the promise of future mediators to improved human and animal health and welfare. Cell cultures and animal models had their glorious dominance in the past and may still be valuable in the times to come. However, veterinary virology will continue to play an important role, because animal viruses can be studied in their natural hosts, and creative ideas may spark from this fortunate situation. To speak with James Richard Broughton: "The only limits are, as always, those of vision". The world-wide community of veterinary virologists must combine its knowledge with that of specialists in immunology, molecular biology, gene therapy, and especially with clinicians. Only then will it be possible to achieve things previously thought to be inconceivable. To conclude with another quote, by Robert Kennedy: I wish that you may dream of things that never were, and ask why not?

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