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Emergency deployment of genetically engineered veterinary vaccines in Europe

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ABSTRACT

On the 9th of November 2015, preceding the World Veterinary Vaccine Congress, a workshop was held to discuss how veterinary vaccines can be deployed more rapidly to appropriately respond to future epizootics in Europe. Considering their potential and unprecedented suitability for surge production, the workshop focussed on vaccines based on genetically engineered viruses and replicon particles. The workshop was attended by academics and representatives from leading pharmaceutical companies, regulatory experts, the European Medicines Agency and the European Commission. We here outline the present regulatory pathways for genetically engineered vaccines in Europe and describe the incentive for the organization of the pre-congress workshop. The participants agreed that existing European regulations on the deliberate release of genetically engineered vaccines into the environment should be updated to facilitate quick deployment of these vaccines in emergency situations.

1. Introduction

The first vaccination in Europe took place in the 18th century, when Europe was plagued by severe outbreaks of rinderpest, a disease caused by the deadly rinderpest virus (RPV). A Dutch farmer and cattle trader named Geert Reinders (1737–1815) observed that calves born from cows that had survived rinderpest were less susceptible to the disease. We now know that this so-called "passive immunity" resulted from the intake of maternal antibodies via colostrum. Moreover, Reinders described that once these calves were inoculated with virulent RPV, they developed only mild clinical symptoms and subsequently became immune to the virus. These first vaccination experiments were described in letters directed to the Royal Society of London in 1776. Twenty-two years later, Edward Jenner (1749-1823) reported that humans could be protected from the highly deadly smallpox (variola) virus by "variolation" with the cowpox virus, a close relative of the variola virus that is non-pathogenic to humans [1]. Later, cowpox was replaced by the related vaccinia virus. The discoveries by Reinders and Jenner contributed to the development of live vaccines that ultimately facilitated the global eradication of two devastating infectious diseases of human and animals; smallpox in 1980 and rinderpest in 2010 [2–4]. Today, after more than two centuries of vaccine research and development, numerous viral diseases can be controlled by vaccination and some are even targeted for global eradication, such as polio encephalomyelitis in the human field [5] and sheep and goat plague (peste des petits ruminants) in the veterinary field [6].

Live-attenuated vaccines against viral diseases of livestock are classically developed by carrying out passages of the viruses in heterologous hosts or cell culture, resulting in the accumulation of attenuating mutations or deletions in the viral genomes. Although numerous vaccines were developed using these methods, the safety of such vaccines needs to be determined empirically and is often not understood at the molecular level. Consequently, these vaccines may suffer from concerns about genetic stability and reversion to virulence. The availability of reverse genetics systems to introduce precisely defined mutations into viral genomes is therefore considered a milestone in veterinary medicine. Already in the early 90's, genetic engineering was used to create a vaccinia virus expressing the rabies virus glycoprotein. After this vaccine was found to be stable and safe for target and non-target animals, it was successfully applied as a bait vaccine in an extensive open field trial [7,8]. Around the same time, genetic engineering was used to attenuate Suid herpesvirus 1, the causative agent of the economically important Aujeszky's disease of swine [9]. The resulting vaccine virus was successfully used to eradicate Aujeszky's disease from The Netherlands [10].

Genetic engineering also enabled the development of multivalent vaccines. A virus that was used for such approach is the herpesvirus of turkey (HVT) [11,12]. HVT is an apathogenic virus that protects chickens against Marek's disease virus, which is caused by the related herpesvirus Gallid herpesvirus 2 and was used to develop bivalent vaccines that also protects against infectious bursal disease virus [13], Newcastle Disease virus [14,15], infectious laryngotracheitis virus [16] or influenza A virus [17–20]. A final example of a multivalent vaccine is based on an attenuated myxomavirus that expresses the capsid protein gene of the calicivirus rabbit hemorrhagic disease virus. This vaccine provides protection from myxomatosis and rabbit hemorrhagic disease, two highly deadly diseases of rabbits [21]. Another highly successful vaccine platform is based on the Canarypox virus (family *Poxviridae*, genus Avipox). Examples of commercially registered vaccines include vaccines for the protection of horses against influenza and

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West Nile virus, cats against rabies and feline leukaemia virus and dogs and ferrets against canine distemper virus [22].

Until today, two vaccines based on genetically engineered RNA viruses were registered in Europe, which are both based on genetically engineered bovine virus diarrhoea viruses (BVDV). The first vaccine contains two genetically engineered BVD viruses that protects cattle against BVDV types I and II [23]. The second vaccine that was recently marketed comprises an attenuated BVDV virus that expresses the E2 protein of classical swine fever virus [24]. Of note, the latter vaccine nicely exemplifies how genetic engineering can be used to develop vaccines that enable Differentiating Infected from Vaccinated Animals (DIVA).

Apart from veterinary vaccines that are already registered, numerous genetically engineered virus-based vaccines are currently in development in Europe. This not only includes live viruses, but also so-called "replicon" particles [25,26]. Replicon particles phenotypically resemble the viruses from which they were derived, but lack (part of) a gene that is necessary for the production of progeny particles [27]. These particles are capable of infecting target cells of the vaccinated animal, but are incapable of spreading from the initial site of infection. By this feature, replicon particles are aimed to combine the efficacy of live vaccines with the safety of inactivated vaccines. Recently, replicon particle-based vaccines targeting porcine epidemic diarrhea virus or avian influenza virus were developed and were granted conditional licenses in the US [28,29].

The availability of platform-based vaccines that are registered in Europe would suggest that these platforms could be used to respond to epizootics within months or even weeks after onset of an outbreak. For example, an incursion of a highly pathogenic and fast-spreading strain of avian influenza virus could be efficiently counteracted by a vaccine based on HVT expressing the hemagglutinin (HA) protein [17]. Preferably, the HVT, or similar vaccine vector, expressing a given influenza virus HA protein is already registered in Europe, which can be updated to fit a novel outbreak strain in a well-defined fast-track process. Unfortunately, within the current legislation, such an emergency response is not possible.

To discuss which innovations are needed in regulatory procedures to enable emergency vaccination with genetically engineered vaccines in Europe, a workshop entitled "From Agent Identification to Vaccine Supply" was organised preceding the World Veterinary Vaccine Congress of 2015, in Madrid, Spain. The workshop was attended by scientists involved in veterinary vaccine development, representatives from leading pharmaceutical companies, regulatory experts and representatives from the European Medicines Agency (EMA) and the European Committee (EC). The use of existing platforms that are already registered in Europe was discussed as well as promising novel technologies. To enable rapid deployment of these vaccines in response to future epizootics, significant innovations in European regulations are required. We here review the *status quo* of the European regulatory arena and propose innovations in regulatory procedures to enable surge vaccine deployment in response to rapidly spreading epizootics in Europe.

2. Current European regulations for genetically engineered vaccines

Since the late 1980's, the European Union (EU) has provided regulations for new technology-based vaccines, including genetically engineered vaccines, starting with Council Directive 87/22/EEC of 22 December 1986. This directive covered the approximation of national measures relating to the placing on the market of high-technology medicinal products, particularly those derived from biotechnology [30]. Later, in 1990, a more specific directive was issued (90/220/EEC of 23 April 1990 [31]), that dealt with the deliberate release into the environment of genetically modified organisms (GMOs) for human and veterinary vaccines. This directive was finally replaced by Directive 2001/18/EC, which describes regulations regarding the release of genetically engineered organisms into the environment [32]. Of note, this directive was amended by Directive 2008/27/EC [33]. The scope of Directive 2001/18/EC, as amended, covers all "*. . . organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination*", where organism means "*. . . any biological entity capable of replication or of transferring genetic material*". Furthermore, the processes used to modify (alter) the genetic material must use: *(1) recombinant nucleic acid techniques involving the formation of new combinations of genetic material by the insertion of nucleic acid molecules produced by whatever means outside an organism, into any virus, bacterial plasmid or other vector system and their incorporation into a host organism in which they do not naturally occur but in which they are capable of continued propagation; (2) techniques involving the direct introduction into an organism of heritable material prepared outside the organism including micro-injection, macro-injection and micro-encapsulation; (3) cell fusion (including protoplast fusion) or hybridisation techniques where live cells with new combinations of heritable genetic material are formed through the fusion of two or more cells by means of methods that do not occur naturally (same directive Annex I.A. part 1).* The interpretation of these definitions has been a matter of debate. For example, a virus or replicon particle resulting from these processes, even when it is not capable of spreading beyond the inoculation site of the vaccinated animal, might fall within the scope of this directive as it brings (or 'transfers') genetic material to the host cell, allowing the expression of antigen(s) that will trigger protection against a specific disease. Indeed, European authorities previously decided that canarypox-based vaccines, which are replication-deficient in mammals, fall under the scope of Directive 2001/18/EC. We therefore expect that also replicon-based vaccines fall within the present scope of the Directive. Renewed discussions on this point will be useful.

The directive gives all instructions to applicants to request a permit for carrying out field trials needed for the registration procedure. For this, the applicant company must prepare a dossier containing a technical file with information relating to: (1) vaccine construct, (2) conditions of release of the vaccine and the target receiving environment, (3) interactions between the vaccine and the specified environment, (4) how to manage the vaccine once introduced into the field with respect to control, remediation methods, waste treatment, and emergency response plans. In addition, an environmental risk assessment must be carried out by the applicant, following prescribed guidelines (Annex II, III and VII of Directive 2001/18/EC, Commission Decision 2002/623/EC [34]). Furthermore, a summary document should be provided (same Directive and Council Decision 2002/813/EC [35]).

The dossier is submitted in the country or countries where the applicant desires to test the vaccine in the field. Subsequently, the vaccine will be assessed by the national competent authorities of each country, which are usually represented by a specific committee of experts in genetically engineered organisms; this committee is a separate entity from the veterinary regulatory authorities. The EC and all other member states are kept informed during this procedure and are entitled to intervene if necessary. Furthermore, the assessment includes consultation of the public. Once this permit is granted by the committee, the applicant can request the other permit needed for testing any veterinary vaccine in the field, which is granted by the veterinary registration authorities. Subsequently, completion of the field trials and finalizing the registration dossier are the final steps prior to submission of the European-wide registration procedure to the EMA. Indeed, all

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