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THE SAFETY OF VETERINARY IMMUNOLOGICAL PRODUCTS IN EUROPE

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Abstract

Vaccination is a standard procedure that stimulates the immune response against microbes. In regards to animals, an indication for vaccination is usually to protect their health as well as economic factors. The purpose of immunoprophylaxis is to reduce the susceptibility of animals to infections by stimulating immunity. Safety and effectiveness of vaccines is dependent among others, on its manufacture process and qualitative composition, with particular emphasis on adjuvants. As public health and animal health are interrelated, it is an important issue. This review article is based on an analysis of the current situation related with veterinary vaccines safety on the European market.

Introduction

Currently in human and veterinary medicine, a lot of attention is paid to proper nutrition and prophylaxis as a way to improve health outcomes. This strength is mostly referred to as immunity – the ability to fight infection. At the same time, animal owners and breeders are concerned about whether the use of vaccines is safe and beneficial. Stimulating the immune response against microbes by vaccination is the most effective method for protecting against infections (BASTOLA 2017, NICHOLSON 2016). Vaccines were used long before the mechanisms of immune protection became known. Today, vaccination is a standard procedure in animal husbandry due to the fact that in the long term, it not only prevents disease but also

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saves production costs. Continued research in the field of veterinary immunology, adaptation of new technologies has provided molecularly defined, genetically engineered, vector vaccines and others (BULL 2015). There are many well-established types of veterinary vaccines on the market successful against viral, bacterial, protozoan and multicellular pathogens. The development of efficacious vaccines has substantially reduced the impact of the human and animal viral diseases on public health (KNIGHT-JONES et al. 2014).

Immunity

The ability to fight infection is referred to as immunity. The collection of cells, tissues and molecules that mediate resistance to infections is called the immune system. Coordinate reaction of these cells and molecules to infectious microbes is an immune response. Immunity may be induced in an organism by infection or vaccination (active immunity) or conferred by transfer of antibodies or lymphocytes from an actively immunised organism (passive immunity). The purpose of immunoprophylaxis is to reduce the susceptibility of animals to infections by stimulating immunity. This can be accomplished by the use of biological and synthetic immune-boosting vaccines (ABBAS and LICHTMAN 2011, SCHUNK and MACALLUM 2005).

Innate immunity is often considered separate from acquired immune responses. Several distinct activities mediate innate immune defense including: epithelial barriers, antimicrobial serum proteins such as complement, natural antibodies produced by B1 lymphocytes, the activity of cells such us neutrophils, macrophages, dendric cells, natural killer (NK) cells that can lyse virus-infected cells also interferon (IFN), apoptosis and small RNA molecules that interfere with virus replication (MACLACHLAN and DUBOVI 2010). Adaptive immunity includes humoral and cellular components. Humoral immunity is mediated principally by antibodies released from B lymphocytes. In addition, dendric cells, macrophages, NK cells and cytokines are all critical to adaptive immune responses. Adaptive immunity is antigen-specific and takes at least several days to develop. This type of immunity is mediated by lymphocytes that possess surface receptors that are specific to each pathogen. Adaptive immunity stimulates long-term memory after infection, meaning that protective immune responses can be quickly reactivated on re-exposure of the organism to the same pathogen (MACLACHLAN and DUBOVI 2010, SHULTZ 1991).

Importance

Vaccination is the most effective way of preventing viral diseases. This concept is considered to have been widely introduced in 1798 by Edward Jenner to protect humans from smallpox. Nearly a century later, the concept was shown by Louis Pasteur and could be used to prevent rabies. In 1950s, a very important segment of vaccine production was developed – live attenuated and inactivated virus vaccines (WEISS and ESPARZA 2015). In Table 1, we can observe the dynamics of some infectious diseases and population data in Europe. Analysing the number of born infants and the number of surviving infants, significant improvement is noticeable. In year 1980: born – 20 197 000, surviving – 13 140 000 and in year 2016: born – 11 183 000, surviving – 11 085 000 (Table 1).

Table 1

Immunization profile in European region

*Population data in thousands	2016	2015	2014	2013	2012	2000	1990	1980
Total population	916'315	913'134	909'962	906'789	903'592	868'538	847'107	797'828
Live births	11'183	11'255	11'307	11'336	11'341	10'267	19'086	20'197
Surviving infants	11'085	11'153	11'201	11'227	11'227	10'083	12'413	13'140
Pop. less than 5 years	56'618	56'815	56'274	56'500	56'098	51'302	64'231	63'920
Pop. less than 5 years	163'867	162'671	161'338	160'041	158'876	172'741	192'275	192'437
Female 15–49 years	213'964	215'146	216'623	218'162	219'667	221'464	208'502	197'358
Number of reported cases								
Japanese encephalitis	0	1	1	0	0	–	–	–
Measles	4'363	25'965	14'176	26'346	27'379	37'421	234'827	851'849
Mumps	20'874	10'027	10'807	35'303	39'072	243'344	–	–
Pertussis	69'490	43'615	43'858	28'170	57'539	53'675	129'735	90'546
Polio	0	2	0	0	0	0	370	549
Rubella	1'471	655	653	39'391	30'579	621'039	–	–
Rubella (CSR)	6	14	28	49	62	47	–	–
Tetanus (neonatal)	0	1	1	0	0	27	69	26
Tetanus (total)	137	122	68	105	208	412	879	1'715
Yellow fever	1	0	0	0	0	0	–	–

Source: World Health Organisation (WHO), http://www.who.int/immunization/monitoring_surveillance/data/en/, access: 16.03.2018.

Currently, modern 'new generation' vaccines are also being produced through various forms of recombinant DNA and related technologies. The majority of large-scale production for use in animals continue to include either a live-attenuated or inactivated virus (MACLACHLAN and DUBOVI 2010).

Live-attenuated virus vaccines can be produces from naturally occurring attenuated viruses, introduced by Jenner in 1798 for the control of human smallpox, utilised cowpox virus, a natural pathogen of cow. The same principle has been applied to other diseases – like Marek's disease, where a vaccine was produced using related herpesvirus of turkeys or the protection of piglets against porcine rotavirus using a vaccine derived from bovine rotavirus (MACLACHLAN and DUBOVI 2010). Live vaccines can also be prepared by attenuation of viruses by serial passage in cultured cells. The cells may be homologous, or more commonly, heterologous host origin. During the accumulate passage in culture cells, viruses typically accumulate nucleotide in their genome, which in turn leads to attenuation. Another possibility is attenuation of viruses by serial passage in heterologous host. For example, rinderpest and classical swine fever viruses were each adopted to grow in rabbits. After serial passage viruses become sufficiently attenuated to be used as vaccines. Viruses can be also passaged in embryonated eggs. Live vaccines can be produced by a selection of cold-adapted mutants and reassortants (MACLACHLAN and DUBOVI 2010). Live viral vaccines have played a successful role in disease control and eradication. Eradication of rinderpest virus from the globe is believed to have been dependent on the use of the "Plowright" vaccine (MEEUSEN et al. 2007).

Inactivated virus vaccines are usually made from a virulent virus; chemical or physical agents are used to destroy infectivity while maintaining immunogenicity. Such vaccines are considered to be safe. The most commonly used inactivated agents are formaldehyde, β-propiolactone and ethylenimine (MACLACHLAN and DUBOVI 2010). Inactivated vaccines are generally more stable and do not post risk of reversion to virulence compared to live vaccines, but they can be less protective due to the inability to activate cytotoxic T cells (MEEUSEN et al. 2007). Consequently, inactivated vaccines generally require strong adjuvants and sometimes more than one injection to induce the required level of immunity. Although inactivated vaccines are considered to be safe, adjuvanted vaccines post a greater risk of causing autoimmune disease, allergic disorders and vaccine injection site sarcomas (MEEUSEN et al. 2007).

Another type of vaccines is those produced by recombinant DNA methods and related technologies. It is a relatively recent technology of vaccine production. This immunological product can be produced by attenuation of viruses by gene deletion or site-directed mutagenesis. Gene

deletion is especially feasible with large DNA viruses that carry a significant number of genes that are not essential for replication in cultured cells. The ability to identify and selectively delete genes from a pathogen has allowed the development of "marker vaccines", that combined with suitable diagnostic assays allow to differentiate animals infected from vaccinated. It is possible by differentiation of antibody responses induced by the vaccine from those induced during infection with a wild-type virus. Such Differentiating Infected from Vaccinated Animals (DIVA) vaccines are available for several infectious diseases including infectious bovine rhinotracheitis (IBR), Aujeszky's disease, classical swine fever (CSF) and food-and-mouth disease (FMD) (MEEUSEN et al. 2007).

Naked DNA can be used for immunisation of animals. DNA vaccination involves immunisation with a plasmid encoding an antigen of the pathogen. The plasmid is transfected into host cells via direct injection, or injection with electroporation or gene gun. The gene of interest then undergoes transcription and translation by host cellular machinery, resulting in the production of an antigenic protein that can induce cellular and humoral immune responses. This type of DNA vaccines promotes induction of cytotoxic T cells after intracellular expression of the antigens. They are temperature stable and safe to transport, which can be important for farms located in remote areas or for wildlife vaccines that need to remain in the open area for a prolonged period of time (MEEUSEN et al. 2007, REEDING and WERNER 2009).

Veterinary bacterial vaccines are also of a great value in a prophylaxis against bacterial diseases. There are three general categories of bacterial vaccines – a live vaccine containing a bacteria that can replicate in the host, thereby functioning as an immunogen without causing its natural disease. A subunit or inactivated vaccine is an immunogen that can replicate in the host. Another type is a DNA-based vaccine, it is taken up by cells, in which it directs the synthesis of bacterial vaccine antigens (ELLIS and BRODEUR 2003). Control of zoonotic diseases have had a major role in reduction of some diseases like rabies, rinderpest, brucellosis and others among people (ROTH 2011).

Safety

The most common failures in the effectiveness of vaccination of animals can be linked to: the vaccine losing immunogenicity due to the expiration date or incorrect storage, the vaccine having been administered via a route other than that indicated by the manufacturer, the animal having been vaccinated during the incubation period of the disease, vaccination

having been induced by the transition of a latent form into an overt form of the disease, the animal being vaccinated in a state of immunosuppression, the animal being in a state of reduced immunological reactivity or hypersensitivity to the components of the vaccine, the vaccinated animal being weakened and poorly nourished or under the influence of strong stress, vaccinated animal having a high level of passive immunity provided by the mother, the protective action of the vaccine being overcome by large doses of the pathogen or very virulent strains (KOSTRO et al. 2015).

Safety and effectiveness of vaccines is also dependent on its manufacture process and qualitative composition, with particular emphasis on adjuvants enhancement of the specific immune response. When developing a composition of an immunological veterinary medicinal product, a key element is to choose the right type of adjuvant that will provide induced immunological response, without adverse reactions. In large-scale breeding, to improve protection, adjuvants must be stable and safe so that they do not decrease the growth and reproduction rate. What is more, adjuvant can reduce number of immunization needed, help to reduce the amount of vaccine material or allow vaccination against a selected disease of several species of animals (GERDTS 2015, PARKER 2009).

Adjuvants can act in a variety of ways: as a specific delivery vehicle, targeting a molecule or acing as a depot at the site of injection, to representing a specific danger signal that induces a very particular type of immune response (VIDYASHANKARA et al. 2015). Most often, adjuvants cause some sort of tissue injury, which in effect can lead to engagement of the immune system and its specialised mechanisms, triggering stimulation and activation of innate and adaptive immunity (GERDTS 2015). Sometimes such activation can result in apoptosis or necrosis at the site of injection, local inflammation manifesting in redness, pain, swelling and more rarely, granulomas or sterile abscesses. It can be perceived as a negative value in connection with visual aspects (animal trade, exhibitions, etc.). Adjuvants can also form a depot at the site of injection, which allows slow release of the antigen and more effective antigen uptake. However, this effect is not necessarily beneficial for all adjuvants and in some cases, transport to the draining lymph node seems to promote antigen-presentation more effectively (GERDTS 2015). Nonspecific adverse effects of vaccines and their adjuvants may also include: fever, arthritis, uveitis, anorexia, soreness and lethargy. An overdose of IL-2, a cytokine used as adjuvant may also increase the probability of autoimmune reactions. (SPICKLER and ROTH 2003, PETROVSKY 2016). A good adjuvant can allow the reduction of the dose or of the antigenic concentration, increasing stability and decreasing the price of vaccine (AUCOUTURIER et al. 2001, AROUS et al. 2013).

The most commonly used adjuvants for commercial animal vaccines are: mineral salts, emulsions (oil-in-water, water-in-oil and water-in-oil--in-water), nanoparticles and microparticles, cytokines, saponins, liposomes and archaeosomes, nonionic block copolymers, derivatized polysaccharides, carrier proteins, complement derivatives, bacterial products and their derivatives, toll like receptor (TLR) ligants and small molecules, immune-stimulating complexes (ICOMs), combination adjuvants, e.g.: FCA (water-in-oil emulsion with mycobacteria) (SPICKLER and ROTH 2003).

UE regulations

Legal requirements regarding the safety of vaccines on the EU veterinary market are defined by various directives and regulations. Marketing authorization holders have to comply with those legislations in areas such as pharmacovigilance, applying to vary a marketing authorisation, submitting product data to European Medicines Agency (EMA) and reporting product defects or recalls. European regulatory requirements for veterinary medicinal products are governed by Directive 2001/82/EC, as amended (Directive 2001/82/EC), Commission Regulation (EU) No. 712/2012 of 3 August 2012 amending Regulation EC No. 1234/2008 concerning the examination of variations to the terms of marketing authorisations for medicinal products for human use and veterinary medicinal products (Commission Regulation (EU) No. 712/2012), Commission Regulation (EU) No. 37/2010 of 22 December 2009 on pharmacologically active substances and their classification in relation to maximum residue limits in foodstuffs of animal origin (Commission Regulation (EU) No 37/2010) and Regulation (EC) No. 470/2009 of the European Parliament and of the Council of 6 May 2009 laying down community procedures for the establishment of maximum residue limits of pharmacologically active substances in foodstuffs of animal origin and repealing Council Regulation (EEC) No. 2377/90 and amending Directive 2001/82/ EC of the European Parliament and of the Council and Regulation (EC) No. 726/2004 of the European Parliament and of the Council (Council Regulation (EC) No 470/2009).

After the authorisation for vaccine is granted, before it can be placed on the market, a specific batch has to be tested. Manufacturers incorporate a batch release safety test into their quality assurance monitoring protocols to meet their internal quality standards and to confirm governmental regulatory requirements. These tests are conducted by vaccinating target species animals or laboratory animals with single or multiple doses of the test batch and observing vaccinated animals for sign of local or sys-

temic adverse reactions. These tests serve as a broad-spectrum bioassay to assess the biological properties of each batch of vaccine approved for the market (GIFFORD et al. 2011). Batch release safety tests are an important part of a comprehensive quality assurance monitoring system. Relevant articles of Directive 2001/82/EC, as amended by Directive 2004/28/EC provide guidelines for the Official Batch Protocol Review Certificate (OBPR) and for Official Control Authority Batch Release of Immunological Veterinary Medicinal Products (OCABR). The procedures and guidelines for running OCABR/OBPR apply equally in all Member States (Council of Europe; EDQM).

Veterinary pharmacovigilance concerns monitoring, evaluating and improving the safety of veterinary medicines, with particular reference to adverse events in animals and human beings related to the use of these medicines. The pharmacovigilance system in the European Union (EU) operates with the management and involvement of national competent authorities, the European Commission and the European Medicines Agency (EMA), in collaboration with the marketing-authorisation holders for the medicines (European Medicines Agency; EMA). Continued increase of the number of reports in the central EU database allows for better monitoring and enables the authorities to provide better feedback to the veterinarians on safe and effective use of veterinary medicinal products in the EU. Summary statistics on adverse event reports for centrally authorised products by target species, including reports in humans (reports received between 1 January 2016 and 31 December 2016) are presented in the Table 2.

Table 2

Summary statistics on reports for centrally authorised products by target species

Species	Number of adverse event reports	Number of affected animals
Dogs	11 657	12 312
Cats	3072	3 499
Cattle	1429	52 926
Pigs	615	320 550
Rabbit	582	6 025
Horse	250	399
Sheep	73	875
Chicken	27	875
Goat	15	927 766
Others*	139	148 161
Human	554	554
Total	18 413	1 475 139

*"Other" species include duck, ferret and guinea pig amongst others
Source: European Medicines Agency (EMA).

EMA has developed a number of tools and measures over time, to promote access and availability of veterinary vaccines to the EU market. These tools include: major species and minor species (MUMS) limited markets policy for immunological, scientific advice, Innovation Task Force and Ad Hoc Expert Group on Veterinary Novel Therapies (ADVENT), accelerated assessment, authorisation under exceptional circumstances, contribution to the Disease Control Tools project (DISCONTROOLS) survey, reduced fees for vaccines against epizootic diseases under certain circumstances, with a multi-strain dossier approach (European Medicines Agency; EMA).

Conclusion

Although there are different types of vaccines and the overall manufacturing procedures for immunological products are standard in the medical industry, there are still important differences between vaccination practices in human and veterinary medicine. First off, all the economic constraints are generally of less importance in human medicine. Veterinary vaccines include about 23% of the worldwide market for animal products. The European veterinary vaccines market is poised to attain a Compound Annual Growth Rate (CAGR) of 4.97% from 2015 to 2020 (PETTERS 2018). The major goals of veterinary vaccines are to improve the health and welfare of companion animals as well as a cost-effective production of livestock and epidemic prevention of zoonotic diseases. Veterinary vaccines can be an efficient tool in reducing the need to use antibiotics in animal husbandry. There is also the issue of safety of immunological products and adverse reactions. Nevertheless, veterinary vaccines have had a significant impact on public health, through increasing safety of the products of animal origin and prevention of infectious diseases transmission between domestic and wild animals, and in an animal-to-human relation.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of the article.

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