

THE OIE'S ROLE IN CONTROL OF LIST A AND EMERGING DISEASES

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The OIE is the international standard-setting organization for animal health and zoonoses. It addresses the control and prevention of important and emerging diseases primarily by collecting and disseminating relevant animal health information worldwide, and by adopting and publishing sanitary standards and recommendations for animals and animal products. The OIE's Information System is aimed at promoting a transparent knowledge of the global animal disease situation, crucial for safe trade decision making. It operates an early warning system which is based on official reports from Member countries, and complemented by an active search and verification of unofficial information. The system requires notifications, and provides reports in several degrees of urgency, determined by the nature of the disease agent and the epidemiological characteristics of the outbreak. For the purpose of harmonization of international trade measures, and in order to prevent discriminatory actions by importing countries, the WTO-SPS requires countries to base their import decisions on science-based international standards.

The Animal Health Code Commission is the specialized committee within the OIE responsible for developing and updating the Terrestrial Animal Health Code, containing all animal health standards for mammals, birds and bees. The disease-specific standards provide recommendations for the safe trade of animal and animal products, making reference to the various possible health status of the exporting country or zone. The Code also provides recommendations on horizontal subjects, such as risk analysis, regionalization, surveillance and monitoring and evaluation of veterinary services. In addition to the Code, through the work of the Standards Commission, the OIE provides recommendations on methods for the diagnosis and prevention of diseases, defines standards for biologic products, vaccines and diagnostic preparations. The presentation will address the mechanism for developing and revising these important trade standards, as well as how Member countries and their interested stakeholders can participate in the standard-setting process.

FAO'S EMERGENCY PREVENTION SYSTEM FOR TRANSBOUNDARY ANIMAL AND PLANT PESTS AND DISEASES (EMPRES) AND THE CONTROL OF TRANSBOUNDARY ANIMAL DISEASES

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Globalisation makes disease eradication and maintenance of diseases free zones increasingly cumbersome. Such diseases threaten global animal agriculture, animal protein food security, food safety and public health. In order to safeguard sustained livestock development in developing countries and permit legitimate participation of these countries and the poor communities within such countries in formal trade, and diminish the risk of epizootics to livestock farming in industrialised countries, the international community needs to take lead from both *World Food Summits* (1996 and 2002) calling for internationally co-ordinated measures for the prevention and progressive control of TADs. The vision of the EMPRES, launched by FAO, is to promote the effective containment and control of the most serious epidemic livestock diseases by progressive elimination on a regional and global basis through international co-operation involving *early warning, early reaction, enabling research* and *co-ordination*. EMPRES distinguishes and emphasises that *vaccines* are different from *vaccination*. The use of vaccines can only occur in a suitable environment – acceptable, easy to administer, inexpensive, early induction and long duration of immunity and ideally allow for the diagnostic discrimination between immunisation and infection, and most important: they reach the rural grazing lands of the pastoralists in proper condition. Of paramount importance would be the international standardisation of vaccines with a clearing house for high quality vaccines for use in the developing world and elsewhere. Biotechnology offers hope for vaccinology, but these endeavours must be acceptable in a world extremely cautious on the use of genetically modified organisms.

A SURVEY OF VACCINES PRODUCED FOR OIE LIST A DISEASES IN OIE MEMBER COUNTRIES

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A survey was conducted to determine the availability, country of origin, and manufacturer of vaccines for all Office International Des Epizooties (OIE) List A diseases. A questionnaire was sent by the OIE to the Chief Veterinary Officers of all OIE member nations. The form, written in English, French, or Spanish, asked whether the country made any vaccines for OIE List A diseases. For each vaccine, contact information was requested for the manufacturer, as well as information on the strain of organism used in the vaccine, the type of product (live, killed, or subunit), and the adjuvant used, if any. Responses returned to the OIE were forwarded to the authors. When alternate contact information was available for non-responding countries, a follow-up survey was sent. In some cases, additional information or clarification was also requested from responding nations. Sixty-one OIE member nations responded to the questionnaire, either initially or on follow-up. The authors did not attempt to gather information on the safety or efficacy of the vaccines in this survey. Anyone interested in using these vaccines is encouraged to request that information from the manufacturer. Contact information for each manufacturer is included. A large number of classical swine fever, foot and mouth disease, and Newcastle disease vaccines were found. A limited number of vaccines were also located for African horse sickness, bluetongue, contagious bovine pleuropneumonia, highly pathogenic avian influenza, lumpy skin disease, peste des petits ruminants, rift valley fever, rinderpest, sheep and goat pox, swine vesicular disease, and vesicular stomatitis. No African swine fever, or swine vesicular disease vaccines were found. Experimental vaccines are not included in this survey. The Institute for International Cooperation in Animal Biologics (IICAB), an OIE Collaborating Center for Diagnosis of Animal Disease and Vaccine Evaluation in the Americas is seeking funding to expand, maintain, and continuously update this database.

INTERNATIONAL STANDARDS FOR VACCINES FOR LIST A DISEASES

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The OIE has been identified as the competent international organization for developing international standards, guidelines and recommendations related to animal diseases and zoonoses. The OIE International Animal Health Code establishes standards for international trade including vaccine requirements for various diseases. The OIE Standards Commission is tasked with establishing standards for veterinary vaccine production and testing which are published in the Manual of Standards for Diagnostic Tests and Vaccines every 4 years. The Manual contains introductory chapters on sterility, principles of veterinary vaccine production, and the role of official bodies in regulation. Each individual disease chapter addresses requirements for vaccine production and standardization. The Standards Commission utilizes input from disease experts to address issues regarding the use of vaccines including strain recommendations, safety testing and potency testing. In addition, companion diagnostic tests or reference reagents are required to be standardized by the criteria established by the Standards Commission with input from disease experts. Examples of OIE's role in addressing List A disease vaccine issues will be presented.

REGULATORY CONSIDERATIONS FOR EMERGENCY USE OF NON-USDA LICENSED VACCINES IN THE UNITED STATES

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The Virus-Serum-Toxin Act of 1913 (21 US Code 151-159) provides the legal basis for the regulation of veterinary biologicals in the United States and the United States Department of Agriculture. The Center for Veterinary Biologics (CVB) has the regulatory authority for the issuance of licenses and permits for such products. The law was intended to establish standards and control the importation of products into the United States and the distribution of products interstate assuring the purity, safety, potency, and efficacy of veterinary biological products. Administrative regulations and standards appear in the Title 9, Code of Federal Regulations, Parts 101-118, with additional program guidance found in CVB Notices, Veterinary Services Memoranda, General Licensing Considerations, and other guidance documents. Prelicensing data evaluation procedures are designed to assess the purity, safety, potency, and effectiveness of each product and support all product label claims. In order to fulfill these criteria, data from all phases of product development are evaluated against these key elements. Under the standard licensing process, this spectrum of evaluation includes complete characterization and identification of seed material and ingredients, laboratory and host animal safety and efficacy studies, stability studies, and postlicensing monitoring of field performance. This comprehensive evaluation may not be possible during the emergence of a new animal disease. While there are no specific regulations addressing the licensing standards of products for an emerging animal disease, there are mechanisms that allow for the availability of products in an emergency animal health situation. These mechanisms include autogenous biologics, conditional licenses, experimental and emergency use authorizations, and the importation of products in use elsewhere in the world. Preapproved vaccine banks provide an additional mechanism. Historical examples of emerging animal disease events in the United States will be used to illustrate the regulatory considerations for each type of product authorization.

REGULATORY CONSIDERATIONS FOR EMERGENCY USE OF VACCINES IN THE EUROPEAN UNION

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From a regulatory perspective foot-and-mouth disease vaccines represent a special case due to the number and antigenic diversity of strains that might be used alone or in combination within the context of an authorisation.

New guidelines have been developed proposing that an FMD vaccine should be defined as a formulation of ingredients including defined amounts of one or more antigens that varies only in the number and types of antigen present. These new guidelines are in line with those previously proposed for equine influenza vaccines. Slaughtering policies being less and less popular in the European Union, there is a tendency to use so-called marker vaccines associated with a companion diagnostic test. Such methodology has already been used for vaccination against pseudo-rabies and infectious bovine rhinotracheitis. Sub-unit marker vaccines against classical swine fever have also been developed; such kind of vaccines are also envisaged against foot-and-mouth disease; it would permit, if satisfying defined criteria, to distinguish vaccinated from infected animals.

VACCINES AND COMPANION DIAGNOSTIC TESTS FOR FOOT-AND-MOUTH DISEASE VIRUS

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Vaccination constitutes an important control policy for foot-and-mouth (FMD) disease eradication programs and for free regions that decide to use immunization as a control measure after a recent introduction of the disease. Taking into account that vaccination protects against disease but not necessarily against persistent infection, availability of tools to identify subclinical infection irrespective of vaccination condition of the herd is of utmost importance. This is particularly relevant under the new vision of OIE for recognition of FMD-free regions that considers not only absence of clinical disease but also of infection

In 1987 a Continental Program for Eradication of FMD in the Americas was launched. Since then, and in support of the surveillance activities to assess the progress of the Program, the search for diagnostic tools to evaluate subclinical FMD viral activity in animal populations, irrespective of vaccination was strengthened at PANAFTOSA, PAHO/WHO. This presentation gives an overview of the 14-year experience gathered during validation and wide field application of a new diagnostic system for use to detect residual FMD viral activity, in regions under intense vaccination campaigns in South America.

Our approach was based on the detection of antibodies against non-capsidial proteins that integrate the replication complex which, in principle, are only induced during infection, and not upon systematic immunization with conventional BEI inactivated vaccines. An immunoenzymatic system was implemented with recombinant non-capsidial proteins as serological probes consisting of an I-ELISA 3ABC as a screening test, followed by confirmation of suspect samples by Western blot (EITB) using 5 recombinant antigens (3A, 3B, 2C, 3D and 3ABC). This allowed us to attain the high sensitivity required for recognition of persistently infected animals, without compromising the specificity needed for use in low prevalence regions in order not to compromise the predictive value.

Among the variables that could affect the application of the system, vaccine interference was a major concern. Vaccines made from partially purified and fully inactivated FMD virus (FMDV) particles may contain variable quantities of some or all of the non-capsidial proteins that upon proper presentation conditions might induce an antibody response in immunized animals. Therefore, experimental and field models were designed to assess whether vaccination under certain conditions could confuse the status of the herd in terms of discrimination between infected and vaccinated animals. Included were: experimentally persistently infected animals followed over time with or without vaccination; animals immunized under various vaccination and revaccination conditions with vaccines from different sources; extensive field samplings under vaccination, representing different epidemiological situations in various countries of South America; and follow-up of outbreaks over time subjected to systematic vaccination. In overall, it was concluded that vaccination is not expected to induce antibodies against non-capsidial proteins that could compromise the interpretation of serosurveillance. Proper criteria for application of the system will be discussed.

The wide experience assembled in PANAFTOSA through the application of the I-ELISA 3ABC / EITB system for use in FMD surveillance demonstrates the effectiveness of this system for assessing risk of persistent viral activity within a herd and confirmation of its absence in a population, regardless of vaccination status. In addition it gave input for understanding the significance of persistent viral activity under the field conditions in South America. Recently the OIE has prescribed the I-ELISA 3ABC /

EITB system for the identification of animals infected with FMDV irrespective of vaccination during serosurveillance.

ENGINEERING BETTER VACCINES FOR FOOT-AND-MOUTH DISEASE

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Over the past 50 years vaccines derived from inactivated preparations of foot-and-mouth disease virus (FMDV) have been used as part of programs that have reduced the number of FMD outbreaks in many areas of the world. Although efficacious and safe, the current vaccines suffer from drawbacks. One of these drawbacks is that the immune response to the vaccine interferes with the ability to detect vaccinated animals that have subsequently become infected and could carry and shed the virus, creating an obstacle to re-instating disease-free status to countries/regions that vaccinate to control outbreaks. There are multiple diagnostic tests available that can identify animals that have been infected with FMDV by detection of antibodies to viral non-structural proteins (NSP) that are present in low concentration in traditional vaccines and are poorly immunogenic in vaccine preparations. However, these tests are not 100% reliable, producing the possibility that FMDV-carriers present in vaccinated populations could escape detection and cause further outbreaks. To overcome this problem, we have developed a new generation of vaccines that express a subunit of the virus, in the absence of one of the most immunogenic NSPs, 3Dpol. The subunit antigen we have selected is the entire viral capsid, known as an "empty viral capsid", which appears to contain all of the relevant antigenic sites of the traditional vaccine antigen. Empty capsid vaccines can be delivered in a variety of ways, but we have found that production of capsids in situ by a recombinant replication-defective human adenovirus is the most effective, and we have used this strategy to create vaccines that can protect pigs from challenge with virulent animal-derived FMDVs. The second problem with traditional vaccines is that they do not induce protective immunity quickly, a drawback shared by our adenovirus-vectored empty capsid product. To overcome this problem, we have developed a prophylactic antiviral treatment that consists of a replication defective adenovirus encoding a type I interferon (IFN), based on research showing that IFN is an important controlling factor in FMDV replication in animals. Administration of this IFN-encoding adenovirus can protect swine from FMD as early as one day post-administration. A combination of this antiviral treatment and the empty capsid subunit vaccine should induce rapid and complete protection from FMD, and provides for the implementation of more sensitive diagnostic tests to identify vaccinated animals that have become infected, and could become asymptomatic carriers of the disease.

INEXPENSIVE VACCINES AND RAPID DIAGNOSTIC KITS TAILOR-MADE FOR THE GLOBAL ERADICATION OF RINDERPEST

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Rinderpest is an acute and highly contagious viral disease of ruminants, often resulting in greater than 90% mortality. Previously, we have developed first (Yilma, et al., *Science* 242: 1058-1061, 1988) and second generation (Giavedoni et al., *Proc. Natl. Acad. Sci. U.S.A.* 88: 8011-8015, 1991) vaccinia virus recombinant vaccines (vRVFV) which provided complete protection against the rinderpest virus (RPV). vRVFV also provided complete protection against peste des petits ruminants (PPR) in goats (Jones, et al., *Vaccine*, 11:9, 961-964, 1993). We have demonstrated that these recombinant vaccines are safe even for immunodeficient mice and rhesus macaques with acquired immunodeficiency syndrome (AIDS). We have now constructed a third generation recombinant vaccinia virus vaccine (v2RVFV) that expresses both the fusion (F) and hemagglutinin (H) genes of RPV under strong synthetic vaccinia virus promoters. v2RVFV-infected cells express high levels of the F and H glycoproteins and show extensive syncytium formation. Cattle vaccinated intramuscularly with as little as 10³ pfu of v2RVFV and challenged one month later with a lethal dose of RPV were completely protected from clinical disease; the 50% protective dose was determined to be <10² pfu. Cattle vaccinated with v2RVFV did not develop pox lesions or transmit v2RVFV to contact animals. Intramuscular vaccination of cattle with 10⁸ pfu of v2RVFV provided long-term sterilizing immunity against rinderpest. In addition to being highly safe and efficacious, v2RVFV is heat-stable, inexpensive, easily-administered, and allows serological differentiation between vaccinated and naturally infected animals (Verardi et. al., *J. Virol* 76: 484-491, 2002).

To aid in the differentiation between vaccinated and infected animals, we have developed recombinant baculoviruses that abundantly express the RPV and PPRV nucleoproteins (N-RPV; N-PPRV) in insect cells (Sf9) and larvae (*Spodoptera frugiperda*). Crude lysates of infected insect cells or larvae, simply diluted in phosphate-buffered saline (PBS), serve as coating antigens in an indirect ELISA (iELISA) for detection of antibodies to RPV and PPRV. For less than \$1 US, enough viral antigens can be produced in a single larva to test more than 10,000 samples, in duplicate (Ismail et. al., *Virology* 198: 138-147, 1994; 208: 776-778, 1995). The Institut Sénégalais de Recherches Agricoles (ISRA) in Dakar, Senegal has successfully transferred the iELISA technology to more than 30 countries in Africa setting the first example of a model for technology transfer among developing countries leading to self-sufficiency. The iELISA kits have been demonstrated to be highly stable (7 days at 37⁰C), highly sensitive, inexpensive, and simple to use. Consequently, the prospects for developing nations to independently accomplish the global eradication of rinderpest by utilizing v2RVFV in conjunction with the iELISA kits is outstanding (Yilma, *Nature/Biotech.* 8: 1007-1009, 1990).

DEVELOPMENT OF NEW GENERATION RINDERPEST VACCINES

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Rinderpest or "cattle plague" is an economically devastating disease still present in some countries in Africa and Asia which is caused by a morbillivirus (RPV) closely related to *Measles* virus. A global rinderpest eradication campaign (GREP) is in place and its goal is the eradication of the disease by 2010. New vaccines which will enable vaccinated animals to be distinguished from animals that have recovered from natural infection, so called "marker vaccines", are being produced to aid in the eradication programme. We have used reverse genetics, which enables genetic manipulation of the virus' RNA genome through a DNA copy, to alter the virus genome to enable a marker vaccines for RPV to be produced. One candidate vaccine, a chimeric virus in which the nucleocapsid protein (N) gene of RPV was replaced by that from the related morbillivirus *Peste des petits ruminants virus* (PPRV), was produced and tested in cattle (rRPV-PPRN). Vaccinated animals can be distinguished from naturally infected ones using two currently available ELISAs which detect antibodies specific to either the N or haemagglutinin (H) proteins of these two viruses. Vaccinated animals become positive in the PPRV-specific test and negative in the RPV-specific test. The opposite is true for the H protein-specific antibodies. Vaccinated animals that subsequently become infected with wild type virus would become double positive for the N protein antibodies of both viruses. In a further development we have, in addition, introduced a positive marker gene into this virus genome, in this case the jellyfish green fluorescent protein (GFP). This vaccine (rRPV-PPRN-GFP) produces antibodies against the marker protein in vaccinated animals which are absent in naturally infected and recovered animals. The antibody response to the GFP protein depended on its mode of expression. Only a membrane-anchored form of this protein gave a positive antibody response in all animals tested while an internally expressed form failed to elicit an immune response in any of the vaccinated animals, despite very high expression levels of the protein. Some animals vaccinated with a virus expressing a secreted form of GFP responded to the marker protein while others showed no response. These findings have implications for the design of new vaccines based on the rescue of negative strand viruses where either marker proteins or additional immunogenic protein genes from other pathogens are incorporated into their genomes to produce dual vaccines. Another marker protein, namely influenza haemagglutinin (HA) which was strongly expressed on the surface of infected cells but was not incorporated into budded virions, gave a strong antigenic response in all animals vaccinated with this vaccine (rRPV-fluHA).

VACCINE IN THE CONTROL OF PESTE DES PETITS RUMINANTS DISEASE

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Peste des petits ruminants (PPR) is a viral disease of sheep and goats that included in the List A of the International Zoosanitary Code. In many cases it is an acute disease that is characterised by an erosive stomatitis, a catarrhal inflammation of the ocular and nasal mucous membranes, profuse diarrhoea. The mortality may reach up 60-70%. In the sub-acute form that is reported to be more frequent in sheep than in goats, the clinical symptoms are far less severe and in that case, usually the affected animal always recovers. Described for the first time in Côte d'Ivoire in 1942, PPR has been associated for a long time with West African countries. However with the help of new and specific diagnostic, tests the understanding of the geographical distribution of this disease has grown very quickly at the end of 1980's. To its historical endemic West African zone, are added all countries of Central Africa, North East Africa, The Middle East and South Asia. In all these areas, PPR seems to be the major constraint in sheep and goat production. The causal agent is a member of the *Morbillivirus* genus as the rinderpest virus. The closely relationship of these two viruses was demonstrated very early by cross-serological reactions and cross-protections against challenge. In the absence of a homologous vaccine, this property was exploited to control PPR with the rinderpest tissue culture vaccine over than 25 years period. However, with the success of the Global Rinderpest Eradication Programme (GREP), the use of the rinderpest vaccine in all animal species is discontinued. Fortunately, an effective PPR homologous attenuated vaccine was successfully developed late in the 1980s. It is now widely used for the control of PPR in domestic small ruminants. The main inconvenient of this vaccine, as other morbillivirus vaccine, is its poor thermal stability. This may lead to a failure of vaccination campaigns in the hot climate areas and where there are deficiencies in the cold chain to maintain good vaccine titres until delivery of the product to targeted animals. In 2001, Worrall et al. succeeded to improve dramatically the heat stability of the PPR attenuated vaccine by dehydration in the presence of trehalose. The new product, Xerovac, is stable at 45 °c for 14 days. Another success in the development of thermostable PPR vaccine has been the recombinant capripox-PPR vaccine. Poxviruses, known to be heat stable viruses, have been widely used as efficient vectors for delivering heterologous genes that code for immunogenic proteins. In this family of virus, the members of capripox group are non-pathogenic for humans, are very limited in their host range (cattle, sheep and goats) and therefore are less likely to cross the interspecies barrier. Moreover, their geographical distributions cover all areas where PPR is endemic. Those qualities make the capripoxviruses the ideal vector for developing dual vaccine for small ruminants. This has been demonstrated with the newly developed recombinant Capripox-FPPR vaccine: it is effective in protecting goats against both PPR and capripox at a dose as low as 0.1 PFU. Such a vaccine, if proved to be effective in providing a long-term immunity against two diseases of the list A of International Zoosanitary Code, PPR and Capripox, would be of high benefit in the improvement of small ruminants production in many areas of Africa, The Middle East and Asia.

DEVELOPMENT OF IMPROVED VACCINES FOR HEARTWATER

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Heartwater is a tickborne disease of ruminants caused by the intracellular rickettsia *Erlchia* (formerly *Cowdria*) *ruminantium*. The disease occurs throughout Africa south of the Sahara and is also present in the Caribbean, where it poses a threat of spreading to the American mainland. Currently the only practical immunization technique is a 50 year old infection and treatment procedure. Animals are infected with viable virulent organisms in sheep blood and are subsequently treated with tetracycline on elevation of temperature. The procedure is not always effective and cannot be used in non-endemic regions, and because the blood must be stored and transported at -40°C it is unsuitable for application in rural areas.

Attempts to make conventional inactivated or attenuated vaccines have previously met with very limited success. Vaccination with inactivated organisms is relatively effective against a homologous needle challenge, but protection against a heterologous field challenge is very limited. Prolonged *in vitro* cultivation of *E. ruminantium* in its normal host cells has been applied to a number of isolates to induce attenuation, and one West African isolate has been successfully attenuated and engenders good protection against homologous needle challenge with the virulent parent isolate. Again, unfortunately, protection against heterologous field challenge is very limited, and several other isolates have been submitted to the same procedure and have proved to be refractory to attenuation.

The type specimen of *E. ruminantium* is the virulent Welgevonden isolate, immunity to which confers a broad spectrum of protection against other isolates. We have developed two new experimental vaccines based on this isolate, both of which induce 100% protection against homologous needle challenge, and one of which also induces 100% protection against heterologous needle challenge with five other virulent isolates.

An experimental attenuated vaccine has been produced by inducing the *E. ruminantium* organisms to infect a continuous canine macrophage-monocyte cell line by supplementing the culture medium with cycloheximide. After continuous propagation for more than a year, involving more than 125 passages, the cultures produced no disease when inoculated into mice or sheep, and the animals were immune to a subsequent lethal needle challenge with 10xLD₅₀ of virulent homologous organisms. Heterologous needle challenges and field challenges are yet to be attempted.

An experimental nucleic acid (DNA) vaccine has resulted from the identification of an *E. ruminantium* genetic locus which contains genes probably involved in the transport of a nutrient essential for the intracellular survival of the rickettsia. Four genes from this locus were separately cloned in-frame into a DNA vaccine vector and the cocktail was used to immunise sheep. In three separate experiments a subsequent needle challenge with 10xLD₅₀ of virulent homologous organisms showed that the animals were 100% protected. In another experiment 100% protection was also demonstrated against needle challenge with 10xLD₅₀ of each of 5 different heterologous virulent field isolates. Some of the challenged animals exhibited mild heartwater symptoms, with moderately elevated temperatures, but they continued to eat normally and recovered with no apparent ill effects.

Sheep immunised with the DNA vaccine were also exposed to a field challenge in a heartwater endemic area and few animals survived. This suggests either that the local *E. ruminantium* genotypes were very

different from any which were used experimentally, or that needle challenge procedures are not a good model for tick challenge in the field. In contrast, when sheep which had been immunised with the DNA vaccine, and had also survived a virulent needle challenge, were exposed to the same field challenge, many of them survived. Their survival was strongly correlated to their reaction to the previous needle challenge, which appeared to have acted as a vaccine boost. Future developments of these vaccines will be discussed.

CONTAGIOUS BOVINE PLEUROPNEUMONIA VACCINES, PRESENT SITUATION AND HOPES

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Contagious bovine pleuropneumonia is a contagious infection of cattle caused by a mycoplasma, *M. mycoides* subsp. *mycoides* SC (MmmSC). It induces lesions of pleuropneumonia in acute cases and the formation of pulmonary "sequestra" in chronic cases. The disease is prevalent mostly in Africa, where it is responsible for high losses, but it has also been sporadically present in Southern Europe until 1999.

Vaccination is now prohibited in most countries except in Africa.

An empirical "inoculation" procedure was developed as early as 1852 in Europe but it may have been used even earlier in Africa. The inoculation of pleural fluid was performed at the tip of the tail in Europe and on the bridge of the nose in Africa. It conferred a good protection but induced a high number of fatal cases. Various inactivated preparations have been tested in the past with inconclusive results leading sometime to some protection and some other time to a sensitisation of the immunised animals. Such preparations have never been used in the field.

Attenuated MmmSC strains have been developed in the 50ies and used extensively in the field both in Africa and Australia. The best known vaccine strains are called KH3J, T1/44 and T1sr. Vaccination campaigns have succeeded in reducing considerably the CBPP prevalence in these two continents but eradication was achieved, in Australia, only by switching to strict measures of animal movement control and stamping out policy.

The search for new CBPP vaccines has become a major issue for African countries that are facing an increase of CBPP outbreak number. The rationale for this search is based on a better understanding of the mycoplasma pathogenicity that could lead to a targeted attenuation of MmmSC strains. It is also based on a better understanding of the bovine immune response that may be driven to a pathogenic inflammatory response or conversely to a better balanced response leading to protection.

RIFT VALLEY FEVER (RVF) AND THE NEEDS FOR VACCINES

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Rift Valley fever is a mosquito-borne epidemic disease of sub-Saharan Africa with the potential to spread to other areas. It has already caused epidemics in Egypt and on the Arabian peninsula and the potential exists for its transmission wherever sheep or cattle are found with abundant mosquito vectors present. Control of the epidemics has never been seriously attempted, in part because of their explosive nature and in part because of the lack of an appropriate vaccine. Existing vaccines include the live-attenuated Smithburn neurotropic strain (induces fetal abnormalities in sheep; low antibody titers reported in cattle) and inactivated vaccines (low immunogenicity, particularly in sheep; slow onset immunity). It should not be difficult to make an effective vaccine against the virus because RVF is not latent and is readily neutralized by antibody. Neutralizing epitopes are invariant among the many RVF strains tested, are present on one of the glycoproteins, and are sufficient for protection. A few neutralizing monoclonal antibody escape mutants have been studied and are attenuated for mice. There are two live-attenuated candidates, MP-12 and clone 3. In addition prototypic alphavirus replicon vaccines have been prepared, and they have the potential advantage of allowing vaccine immunity to be distinguished from post-infection immunity. Non-replicating constructs have been prepared using either hepatitis B particles as carriers or expressing RVF glycoproteins in baculovirus-infected insect cells. There is an urgent need to develop one or more of these approaches into a practical, field-tested vaccine.

VACCINES FOR LUMPY SKIN DISEASE, SHEEPPOX AND GOATPOX

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Sheeppox, goatpox and lumpy skin disease (Neethling) are pox diseases of sheep, goats and cattle respectively caused by strains of poxvirus, within the genus Capripoxvirus. Strains affecting sheep and goats are not totally host specific, some cause disease in both sheep and goats while others may cause disease in only one species. Those causing disease in cattle appear to be specific for cattle, and this is reflected in the different geographical distribution of lumpy skin disease (LSD) and sheeppox and goatpox (sheep and goatpox) - LSD is confined to Africa while sheep and goatpox is present in Africa north of the equator, and throughout West Asia and India, as far East as China and Bangladesh. Occasionally sheep and goatpox spreads from Turkey into Greece. All strains of capripoxvirus so far examined are antigenically indistinguishable, and recovery from infection with one strain provides immunity against all other strains. Because of this antigenic homology amongst all strains, there is the potential to use a single vaccine strain to protect cattle, sheep and goats.

POTENTIAL FOR DEVELOPMENT OF A VACCINE FOR AFRICAN SWINE FEVER (ASF)

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The current thrust of much ASF research involves identification of factors and mechanisms affecting virus-host interactions, including those responsible for viral pathogenicity and host range specificities. Complete genome sequences of several ASF viruses (ASFV) have revealed a large number of genes that are likely to contribute to ASFV virulence and host range. These include genes and gene families found in variable genomic regions located near the genomic termini, ASFV-specific genes and genes similar to known genes from other viruses or organisms. Genetic and biochemical studies have identified and characterized genes crucial for aspects of virulence and host range. A basic understanding of ASFV interactions with its hosts is beginning to emerge. This provides the information necessary for engineering attenuated or nonreplicating (in the swine host) viral strains as vaccine candidates. In addition, a working understanding of the molecular complexities underlying ASFV-host-vector interactions may suggest novel approaches for disease control that exploit viral host range.

Comparative Genomics of Capripoxviruses: Insights into Pathogenesis and Vaccine Development

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Viruses belonging to the capripoxvirus genus (CaPVs) of the *Poxviridae* are etiologic agents of important domestic animal diseases in the developing world. These include lumpy skin disease virus (LSDV), which causes disease in cattle in central and southern Africa, and sheeppox virus (SPPV) and goatpox virus (GTPV), which cause disease in sheep and goats in northern and central Africa, southwest and central Asia, and the Indian subcontinent. Here we report the genomic sequence and comparative analysis of eight LSDV, SPPV, and GTPV isolates, including five pathogenic field isolates and three attenuated vaccine viruses. All CaPV genomes are approximately 150 kbp and are strikingly similar to each other, exhibiting at least 96% nucleotide identity over their entire length. Wild type LSDV genomes have the most complete coding content of the CaPVs, containing up to 156 putative genes encoding conserved poxviral replicative and structural proteins and proteins likely involved in virulence and host range. SPPV and GTPV genomes are very similar to that of lumpy skin disease virus (LSDV), sharing 97% nucleotide identity. All of the at least 147 SPPV and GTPV genes are present in LSDV; however, nine LSDV genes with likely virulence and host range functions are disrupted in both SPPV and GTPV, including a gene unique to LSDV (LSDV132) and genes similar to interleukin-1 receptor, myxoma virus M003.2 and M004.1 (two copies each), and vaccinia virus F11L, N2L, and K7L. The absence of these genes in SPPV and GTPV suggest a significant role for them in bovine host range. Overall, CaPV genomes contain specific nucleotide differences suggestive of a phylogenetic distinction correlating to host species. Relatively few genomic changes may account for CaPV viral attenuation, as SPPV and GTPV vaccine viruses contain 71 and 7 genomic changes compared to their respective field strains. Notable genetic changes include mutation or disruption of genes with predicted functions involving virulence and host range, including kelch-like and superoxide dismutase-like proteins in LSDV, two ankyrin-repeat proteins in SPPV and three kelch-like proteins in GTPV. These comparative genomic data provide greater insight as to the genetic basis for CaPV virulence and host range and they indicate the close genetic relationship among CaPVs, suggesting that SPPV and GTPV are distinct and likely derived from an LSDV-like ancestor.

NATURE AND DURATION OF PROTECTIVE IMMUNITY TO BLUETONGUE VIRUS INFECTION

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Vaccination has been a most successful methodology to combat diseases in man and livestock. Most of the current viral vaccines are prepared using attenuated or inactivated virus. This approach, although useful in many cases, has certain drawbacks. For example, for attenuated live virus vaccine there have been vaccine breakthroughs (Rotavirus vaccine, African horsesickness), or disease caused by incompletely inactivated vaccine (particularly for Foot-and-mouth disease vaccines). Control of Bluetongue virus disease is particularly difficult due to the multiple serotypes of the virus. In addition, viral genome is made up of 10 segments allowing exchanging the genes randomly between different viruses. This may cause generation of infectious virus with mixed genes. Also such live virus vaccine production has to be undertaken in containment laboratories providing added costs both for the production and for the safety and efficacy testing of vaccine lots.

Recent protein expression technology has provided novel approaches to developing intrinsically safe vaccines. The technology involves the synthesis of immunogenic proteins and particles that elicit highly protective immune responses. Successful vaccine development requires systems where the engineered products mimic the authentic proteins, not just in terms of their primary amino acid sequences but specifically in terms of their three dimensional structures, i.e., the products must be as authentic as possible. We have utilized one such protein engineering systems to synthesise individual Bluetongue virus proteins and core- (single coat) and viral-like (double coat) multiprotein structures (VLPs, CLPs) at high level. These engineered particles essentially mimic the virus particles, but do not contain any genetic materials.

The immune responses of these synthetic proteins (subunits) and empty particles (non-replicating) have been tested both *in vitro* virus neutralising tests and *in vivo* animals (BTV susceptible sheep). Based on this initial data, a series of clinical trials have been undertaken using animals (from 50 to 200 sheep in each trial). When one unpurified protein (virus neutralisation protein VP2) was used for vaccination of sheep, 50 µg/dose could afford protection in sheep against virulent virus challenges, while much less of this same protein was needed for complete protection when mixed with the second virus outer coat protein, VP5. In contrast, vaccination with 10 µg VLPs in several trials (each including 100-200 sheep) gave long lasting protection (at least 15 months, maybe longer) against homologous BTV challenge. Cross-protection was also achieved depending on the challenge virus and the amounts of antigen used for vaccination.

Limited vaccination trials with CLPs that contain only the two conserved proteins VP3 and VP7 had also been undertaken against homologous and heterologous BTV challenges. It was clear that CLPs could provide either partial (with only slight fever) or complete protection (depending on dose) against homologous and heterologous virus challenges. Animals showed strong group specific antibody response, but no neutralising antibodies. Since CLPs are conserved across the various serotypes, CLPs could have potential for candidate vaccine which may at least mitigate the Bluetongue disease and inhibit the virus spread.

BTV CLPs and VLPs offer particular advantages as potential vaccines over other systems. The large quantities of CLPs and VLPs can be produced due to the high expression capabilities of baculovirus vectors (produced in serum-free medium), and can be purified using a one-step generic protocol based on the physical properties of the particle. More importantly, these particles are devoid of any detectable amount of insect, or baculovirus proteins or nucleic acids and thus poses no potential adverse effects.

MARKER VACCINES AND COMPANION DIAGNOSTIC TESTS FOR CLASSICAL SWINE FEVER

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The principle of marker vaccination is the differentiation between specific antibodies in animals vaccinated with a marker vaccine and antibodies in animals which recovered from a field virus infection. Therefore a discriminatory test is needed, which is a vital element of the marker vaccination system. Live marker vaccines consist of a virus with a deleted protein and are in use for porcine Pseudorabies virus (PRV) and bovine Herpesvirus (BHV-1). The discriminatory tests (usually ELISA systems) detect antibodies against the deleted protein to identify a field virus infection. The PRV system has been successfully used for eradication programs in Europe and the BHV system is currently in use in Germany.

In contrast, for Classical Swine Fever (CSF) a subunit vaccine (principle of an inactive vaccine) is available, which consists of a single viral protein inducing neutralising antibodies when injected in the host. The discriminatory ELISAs detect antibodies against another viral protein (E^{ms}) indicating a field virus infection.

When used as a prophylactic vaccination, the subunit vaccines induce good immunity against the clinical signs of CSF. As CSF has already been eradicated from many countries the use in these regions can only be contemplated as emergency vaccination after a new introduction of virus. Therefore, a *Large Scale Marker Vaccine Trial* was financed by the EU Commission and organised by the EU Reference Laboratory for CSF in 1999. When tested under the conditions of emergency vaccination, e.g. challenge before full immunity had developed, it was shown, that most CSF challenge infections took a subclinical course with reduced virus shedding. Transplacental transmission in pregnant sows could not be prevented after an application of a single vaccine dose. The most serious deficiencies have been found in the discriminatory ELISAs. Both available tests have shown deficiencies in sensitivity and specificity compared to conventional CSF antibody ELISAs. At the time, when the trial was performed, no confirmatory test was available to verify the result of the discriminatory ELISAs.

Currently two new developments of marker vaccines for CSF are in progress. A chimeric vaccine is based on infectious clones of the conventional live vaccine (C-strain) where a gene is replaced with the corresponding gene of the closely related pestivirus BVDV. The second principle is the construction of a DNA vaccine. Therefore the gene of the appropriate viral protein is cloned into a plasmid and replicated in bacteria. After intramuscular injection the viral DNA is taken into the animals cells and proteinbiosynthesis of the viral protein can start, bringing the immune system of the host animal into contact with the viral protein.

Both new developments are based on the principle of live vaccines whereas the principle of the discriminatory test is still the same. Antibodies directed against the same wild type protein (E^{ms}) will be detected in the discriminatory ELISA.

The most challenging approach is the vaccination of wild life with a marker vaccine. Especially CSF in wild boar is a constant threat for the domestic pig population in some parts of Europe. Currently the wild boar population is vaccinated with C-strain vaccine in two parts of Germany. As the use of conventional vaccines in wild boar implies restrictions to the domestic pig farms a marker vaccine would be ideal. But any form of marker vaccination will have to be administered orally, consequently excluding the inactivated vaccines.

CHARACTERISTICS OF A COMMERCIALY AVAILABLE MARKER VACCINE AND DIAGNOSTIC TEST TO COMBAT CLASSICAL SWINE FEVER

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Though Classical Swine Fever (CSF) has been eradicated in a number of countries; it is still a disease, which occurs almost worldwide. With the modern tools of biotechnology a safe and efficacious marker vaccine based on the E2 protein (major immunogen) of the CSF virus has been developed. This marker vaccine with the complementary testkit allows the differentiation between vaccinated and infected animals, offering new opportunities in controlling the disease. The efficacy and safety of the CSF E2 marker vaccine have been demonstrated in comprehensive trials including 2 week old piglets, fattening piglets and pregnant sows. Three trials are presented here as examples.

1. Prevention of horizontal transmission of CSFV in a vaccinated population

The onset of immunity and the challenge virus spread were evaluated in a transmission trial to determine the “reproduction ratio” (R-value). If $R < 1$ the infection will die out. If $R > 1$ the infection may not die out, and outbreaks may continue.

The experiments involved 5 groups of ten 6-7 week-old SPF pigs. Pigs in four of these groups were vaccinated once IM with a single dose of the CSF E2 vaccine. Pigs in the 5th group served as unvaccinated controls. Five pigs in each vaccinate group were challenged intranasally with 1 ml/pig ($10^{3.5}$ TCID₅₀) of virulent strain Brescia at 7, 10 and 14 days after vaccination, respectively. The other half of the group was separated for one day and then commingled again to serve as contact controls. The unvaccinated control group was handled accordingly. The occurrence of virus spread was evaluated clinically, virologically and serologically employing the differentiating E^{RNS} ELISA.

In the control group directly challenged pigs and contact sentinels died. In the vaccinated group challenged at 7 day interval virus transmission to contacts occurred, which all survived. In contrast in the 10 and 14 day interval groups contact infections did not occur. The R-value was < 1 already at 10 days post vaccination, indicating that horizontal virus transmission could be blocked effectively.

2. Vaccination and challenge of pregnant sows (vertical transmission)

If a pregnant sow gets infected with CSFV the virus may cross the placenta and infect the fetus, with the risk of parturition of persistently infected piglets. The latter can be a major factor in the epidemiology of CSFV, because these animals initially may go unnoticed, and since they are highly viremic, may spread high titers of virus in the environment.

Three groups of 9 conventional CSF-free gilts, were vaccinated once (24 days prior insemination), twice (24 days prior and 17 days post insemination) or not at all to serve as control group. The sows were challenged each intranasally with 5 ml of the Dutch mild virulent strain “Zoelen” containing 10^4 TCID₅₀/ml in the 7th week of gestation. At different timepoints before and after vaccination and challenge body temperatures were monitored, and blood serum samples were taken. Five weeks after challenge the sows were terminated. The fetuses and organs of sows were removed for analysis for the presence of CSF virus.

After challenge no clinical signs of CSF or abortions were observed in sows in any of the groups. At 5 weeks after challenge all control sows had seroconverted, and were negative for CSFV in their organs. In contrast, fetuses of all of these sows were virus positive, indicating transplacental transmission of CSFV. In the once vaccinated group only 1 sow had virus positive fetuses. Thus, 8 of 9 sows in this group appeared to be protected against transplacental transmission. In the twice vaccinated group none of the sows transmitted challenge virus to their fetuses.

3. Vaccination of piglets with maternal antibodies and duration of immunity

In a vaccination campaign maternal antibodies due to vaccination of sows may interfere with early vaccination of piglets. To evaluate whether there is such interference in piglets vaccinated at 2 weeks of age three separate trials were done, of which one is presented.

Fourteen piglets born from sows vaccinated twice with the CSF E2 vaccine were allocated to two groups (G, H) of 7 each. Six piglets from unvaccinated sows were allocated to a third group (I), and further 2 piglets to a 4th (control, J) group. The piglets of groups G and I were vaccinated with one dose at 2 weeks of age. All piglets in the trial were challenged intranasally with 100 LD₅₀ of strain Brescia at 5 weeks of age. To each of the groups 24 hours after challenge two non-vaccinated, susceptible piglets were added as contact sentinels. The piglets were evaluated clinically, virologically and serologically employing the differentiating E^{RNS} ELISA and the IPLA.

E2 antibodies were transferred via colostrum in high titres to piglets derived from vaccinated sows. These antibodies alone were not protective against challenge. Vaccination of maternally immune piglets, however did induce clinical protection against the challenge although it could not prevent the transmission of CSFV to the susceptible sentinels. The vaccinated piglets without maternal antibodies were also protected against CSF, but also in this group transmission of CSFV to a sentinel occurred.

The protection of the piglets vaccinated at 2 weeks of age in presence of maternal antibodies lasted for six months as demonstrated in other trials.

Summary of conclusions

The CSF E2 marker vaccine:

- Induces immunity as early as 10-14 days after single dose vaccination resulting in
- Clinical protection against CSF
- $R < 1$ (within herd)
- Protects sows following two vaccinations against transplacental transmission of CSFV
- Vaccinated pigs can be differentiated from infected by the accompanying testkit

SUITABILITY OF AN E2 SUBUNIT VACCINE IN COMBINATION WITH THE E^{RNS}- MARKER-TEST FOR ERADICATION THROUGH VACCINATION

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Vaccination with live vaccines against Classical Swine Fever (C-strain, GPE-minus strain) has proven to be an efficient tool in the control of CSF. However the antibody response after vaccination cannot be distinguished from the response after infection. Hence it is impossible for authorities to serologically monitor the spread of the CSFV in a vaccinated population.

With the combination of a subunit marker-vaccine, based on the E2 protein, and a marker-test, based on the E^{RNS} protein, it is now possible to distinguish and to screen vaccinated populations for infection.

Porcilis Pesti, the E2 vaccine from Intervet has passed in 2000 the stringent requirements for safety and efficacy of the European Medicine Evaluation Agency and is now licensed in Europe. Efficacy trials with the vaccine have been reported in Europe, United States and Asia (Taiwan).

Double vaccination: Double vaccination with the sub-unit vaccine provides a good or even complete protection against challenge. Double vaccination of pregnant gilts reduces but not completely prevents the trans-placental transmission of the challenge virus.

Single vaccination at 14 and 21 days before challenge: Single vaccination of weaners provided a good protection against challenge at 14 and 21 days post-vaccination. The vaccination almost completely prevented infection of vaccinated contact pigs.

Single vaccination at 10 and 7 days before challenge: After challenge at 10 and 7 days after vaccination, single vaccination provided reasonable protection against challenge at 10 days after vaccination and, to a lesser extent 7 days after vaccination. The vaccination still reduced the infection rate in vaccinated contact pigs, although to a lesser extent in the 7 days group.

Due to partial efficacy at 7 and 10 days and good efficacy at 14 and 21 days after single vaccination, the marker-vaccines are suitable for use in an emergency program. Double vaccination remains the preferred route in endemic areas. The marker-vaccines preclude large scale destruction of healthy vaccinated animals. An efficacious live vaccine most likely would offer protection a few days earlier than the sub-unit vaccine. However these vaccines cannot be used in a “vaccination to live” scenario.

The European evaluation program of the E^{RNS} tests concluded that both tests, at that moment, had too many limitations to be the basis for an emergency vaccination program with the marker-vaccines. Intervet’s test has in the meantime been improved with regards to robustness, sensitivity and specificity. Data on the second version of Chekit CSF Marker will be presented. The improvements no longer preclude the use of the marker-vaccines.

VACCINES FOR AVIAN INFLUENZA

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Various vaccine technologies have been shown experimentally to be effective for immunization against AI and include conventional inactivated oil-based whole AI virus, vectored virus, subunit protein and DNA vaccines. This protection is based upon antibodies produced against the surface glycoproteins, principally the hemagglutinin, but also the neuraminidase. This protection is specific only for individual subtypes of hemagglutinin (H1-15) and neuraminidase (N1-9) proteins. Avian influenza vaccines protect chickens and turkeys from clinical signs and death, and reduce respiratory and intestinal replication of a challenge virus containing homologous hemagglutinin protein. Many of the vaccines are effective if given by single injection and provide protection for greater than 20 weeks. Protection has been demonstrated against both low and high doses of challenge virus. Furthermore, AI vaccines have been shown to provide protection against homologous field viruses with over 10.6% difference in hemagglutinin sequence homology and isolated over 29 years. Currently, inactivated whole AI virus vaccines and a fowl pox-vectored vaccine with AI H5 hemagglutinin gene insert are used commercially in various countries of the world. These vaccines have economic and intense labor disadvantages associated with parenteral administration. However, a recombinant Newcastle disease virus vaccine with an AI hemagglutinin gene insert shows promise as a low cost, mass administered aerosol vaccine. Critical for the use of vaccines in the field is the ability to differentiate vaccinated birds from birds exposed to the field virus. Differentiation is necessary for outbreak surveillance and trade. The use of AI vaccines varies with individual countries and for different AI virus subtypes.

VACCINATION POLICY APPLIED FOR THE CONTROL OF AVIAN INFLUENZA IN ITALY

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During 1999-2001 Italy has been affected by four subsequent epidemic waves of avian influenza caused by viruses of the H7N1 subtype. The first epidemic wave was caused by a low pathogenicity avian influenza virus of the H7N1 subtype that subsequently mutated into a highly pathogenic avian influenza virus, after circulating in the industrial poultry population for approximately nine months. Following the emergence of the HPAI virus, that caused death or culling of over 13 000 000 birds, and the implementation of the measures indicated in Council Directive 92/40/CE, the LPAI virus re-emerged twice.

In order to control the re-emergence of LPAI virus and to develop a novel control strategy, a coordinated set of measures, including strict biosecurity, a serologic monitoring programme and a “DIVA” (Differentiating Infected from Vaccinated Animals) strategy were enforced (Commission Decision 2001/721/CE). The “DIVA” strategy was based on the use of an inactivated oil emulsion vaccine containing the same haemagglutinin (H) subtype as the field virus, but a different neuraminidase (N), in this case an H7N3 strain. The possibility of using the diverse N group, to differentiate between vaccinated and naturally infected birds, was achieved through the development of an “*ad hoc*” serological test based on the detection of specific anti- N1 antibodies.

The control of the field situation was ensured through an intensive sero-surveillance programme aiming at the detection of the LPAI virus, through the regular testing of vaccinated flocks. Serologic monitoring was also enforced in unvaccinated flocks, located both inside and outside the vaccination area. In addition, the efficacy of the vaccination schemes was evaluated in the field through regular testing of selected flocks.

After the first year of vaccination, the epidemiological data collected, indicating that the H7N1 virus was not circulating any longer, was considered to be sufficient by the EU Commission to lift the marketing restrictions on fresh meat obtained from vaccinated poultry (Commission Decision 2001/847/CE).

The experience gathered during the Italian 1999-2001 AI epidemic, suggests that the combination of a “DIVA” control strategy with a territorial monitoring system under official control may represent an effective tool for the control of avian influenza infections in poultry. In addition, the application of a “DIVA” vaccination policy, as opposed to a conventional policy enabled veterinary public health organisations to establish that infection was not circulating any longer, and ultimately resulted in the possibility of marketing meat obtained from animals vaccinated against an OIE List A disease.

EQUINE VACCINE FOR WEST NILE VIRUS

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The West Nile virus (WNV) was first isolated and identified from birds, mosquitoes, and mammals including horses in three states of the northeastern United States in 1999. Since then, WNV infection has been spread to southeastern and mid-western states. In order to meet the urgent need of controlling the WNV infection in equine population, we have developed a killed WNV vaccine. A dose titration study in horses was first conducted to evaluate serum neutralization antibody responses against WNV in horses. Horses were randomized into three vaccinated and one control groups. Horses were vaccinated with the test vaccine at low, median and high dose respectively. All vaccinated horse were administered the test vaccine intramuscularly twice, three weeks apart. Serum samples were collected periodically and were measured for serum neutralization titers using plaque reduction neutralization test. Twelve months after the second vaccination, horses vaccinated with the median dose of WNV vaccine and non-vaccinated control horses were experimentally challenged with WNV. After challenge, horses were monitored for rectal temperature and any clinical signs twice daily for two weeks and once daily thereafter until 21 days post challenge (DPC). Serum samples were collected twice daily for two weeks and once weekly thereafter for detection of viremia. Horses were euthanized and necropsied on 21 and 22 DPC. Cerebrospinal fluid (CSF), spinal cord (cervical, thoracic, and lumbar) and brain (frontal, occipital, medulla oblongata, and brain stem) tissue samples were examined for gross pathology and collected for virus isolation. Nine out of 11 (81.8 %) controls developed viremia after challenge while only one out of 19 (5.3 %) vaccinates had transient viremia. No WNV associated clinical signs were observed in any of the challenged animals throughout the observation period. No febrile responses were observed in any of the challenged horses. No WNV was isolated from any of the tissue or CSF samples collected from any of the challenged horses. Results from this study demonstrate a significant protection (94 % of preventable fraction) against viremia in horses vaccinated with the killed WNV vaccine and the long duration of the protective immunity.

SUITABILITY OF PRESENTLY AVAILABLE VACCINES FOR CONTROLLING THE MAJOR TRANSBOUNDARY DISEASES THAT AFFLICT SUB-SAHARAN AFRICA

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Africa, because of the number and variety of ungulates and haematophagous arthropods that have evolved there, has a plethora of micro- and macro-cellular pathogens that co-evolved with these hosts. These pathogens not only infect the species with which they evolved but also, and usually more dramatically, afflict domestic livestock and, sometimes, humans as well. Furthermore, due to the regular introduction of livestock from other regions of the world over the last few hundred years, pathogens exotic to the continent such as rinderpest virus, have also become established there. African livestock farmers, particularly those who farm with so-called “improved breeds” therefore have a hard row to hoe.

In order to raise livestock successfully in Africa it has been necessary to develop a range of vaccines against infections that for a variety of reasons are now recognised as “transboundary”. In the first 75 years of the last Century vaccines against a number of these transboundary diseases were developed in Africa. Examples include African horsesickness (AHS), rinderpest, lumpy skin disease (LSD), heartwater (cowdriosis) and blue tongue (BT). For other infections such as foot and mouth disease (FMD), contagious bovine pleuro-pneumonia (CBPP), sheep and goat pox (S&GP) and peste des petits ruminants (PPR), vaccines in current use were developed in Europe or the Middle East. The result is that there are vaccines against almost all the major African transboundary diseases, African swine fever (ASF) being an exception. However, the efficacy of these vaccines is variable. There are also financial, technological, logistical and political constraints in Africa that render the control/eradication of major transboundary diseases through the use of vaccines problematic. These continue to impede both productive capacity – resulting in widespread dietary protein deficiency – and the ability to export livestock and their products and therefore limit the contribution of livestock to wealth creation.

These issues – both generic and specific – will be discussed in relation to rinderpest, CBPP, RVF, FMD, LSD, PPR, BT and S&GP. The urgent need for a vaccine against ASF is emphasised.

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**CHALLENGES AND OPPORTUNITIES IN DEVELOPING AND MARKETING VACCINES
FOR OIE LIST A AND EMERGING ANIMAL DISEASES**

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Veterinary pharmaceutical products generated 14.5 billion U.S. Dollars (USD) in worldwide sales in 2000, with biological products contributing 16.2 percent or 2.3 billion USD. The leading biological products were foot-and-mouth disease (FMD) vaccines, with 284 million USD in sales, representing 26.4 percent of the entire livestock biological business. Despite the potential opportunities for the biologics industry, non-vaccination policies and undefined eradication and disease-prevention programs limit the investment the private sector can make in the research and development of vaccines against List A diseases. The focus of the private sector remains vaccines for infectious diseases that impact current domestic herd health management systems. Changing the vaccine paradigm, investing in new technologies, and creating the future by integrating into key alliances with producers, government, and regulatory authorities will be paramount to protecting our poultry and livestock industries against future epidemics and potential acts of bioterrorism.

NEW VACCINES AND THEIR IMPACT ON PROGRAMMES FOR CONTROLLING PIG DISEASES

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Molecular biology and technological advances in DNA (desoxyribonucleic acid) recombination have ushered in a new era in vaccinology.

Historically, vaccines such as those developed against Aujeszky's disease have had a dramatic impact on control and eradication programmes and the use of deleted serological marker vaccines has provided a considerable advance, as mass vaccination is now possible without compromising serological diagnosis. This has enabled vaccinated and infected herds to be pinpointed and the necessary measures applied to prevent the field virus from spreading outside these herds. Secondly, gradual sanitation measures can be implemented with confidence in vaccinated and infected herds, by culling infected sows as required. Such sows are detected through serological screening using the ELISA technique, enabling vaccinated pigs to be distinguished from those that have been vaccinated and are infected.

However, the cost of vaccination must be taken into account when calculating the total cost of a prophylactic treatment.

In the light of past and present experience, it has therefore become possible to develop a strategy for using vaccines to control Aujeszky's disease : so, in countries that have sufficient economic resources to envisage eradication of the infection, there are two possible options.

Firstly, where the prevalence of infection in a given territory is high, or there is a high density of pig herds, mass vaccination with effective deleted vaccines is the only means of reducing prevalence; however, although such measures are necessary, they are not in themselves sufficient to eradicate the infection. Identification, screening and culling of the infected breeding animals seem to be essential for successful eradication combined with systematic vaccination of the animals for at least two years after elimination of the last infected pig. In the latter case, it is advisable to control the movements of piglets, pigs for consumption and breeding animals as much as possible.

Secondly, in regions with a low herd density and low prevalence of Aujeszky's disease, serological screening and the culling of infected breeders or the total slaughter of certain herds, appear to be the most effective, and in some cases the most economical, measures for achieving eradication.

A new generation of vaccines against Classical Swine Fever recently appeared. The serious epizooty appeared in Europe in 1997 raised up a debate on the interest of vaccination to control the spreading of the virus. But the circumstances of the epizooty were particular as 22 herds were already infected when the primary outbreak was identified. So, the use of a serological marker vaccine would not radically alter the basic nature of the problem. The conditions of the use of a vaccine as a tool in an eradication programme are discussed.

THE USE OF EMERGENCY DIVA VACCINES IN THE CONTROL OF LIST A DISEASES

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Many countries are free of one or more list A diseases, and many others have programmes in force that are aimed at eradication of the agents that cause these highly contagious diseases. A country that is free of a list A disease and has adopted a non-vaccination policy against that disease has achieved the highest animal health status, which offers numerous advantages in trade and saves the country often millions of euros a year.

However, the likelihood that list A diseases can again be reintroduced in free countries has not lessened during the last years, as has been shown by recent severe outbreaks of foot-and-mouth disease, classical swine fever and avian influenza in several member states of the European Union. Globalisation in its broadest sense is a main explanatory factor for this threat. Countries must therefore be well-prepared to cope with an unexpected outbreak of a list A disease on their territory. Besides passive and active surveillance, making a rapid diagnosis is of vital importance to prevent a single outbreak from developing into a widespread epidemic, which has enormous social, financial and cultural consequences.

Therefore, more attention should be given to the development of pen-site diagnostic tests that can be applied by the veterinary practitioner.

In the very beginning of such an outbreak, it should also be promptly decided whether an emergency vaccination will be implemented or not. Not doing so will often result in pre-emptive culling of healthy uninfected herds of animals; a policy which will be confronted with more and more public opposition. The decision to emergency-vaccinate is also dependent on efficacy, safety and availability of so-called emergency vaccines, and on the possibility to have these administered to sufficient herds in the neighbouring region in a very short period. If highly efficacious diva vaccines and high-quality elisas to differentiate infected from vaccinated animals would be in place, the decision to emergency-vaccinate and thus to follow a diva strategy, would not be a difficult one. However, there is room for improvement of emergency diva vaccines and the accompanying differential antibody tests for foot-and-mouth disease and classical swine fever. An emergency vaccine should induce a herd immunity in 2-3 days, in that it prevents or reduces transmission of wild-type virus in a herd. As a consequence, such a herd would no longer be a source of dissemination of wild-type virus, which eventually results in restricting the outbreak. Such emergency vaccinations may lead to healthy 'carriers' of wild-type viruses. However, it is not clear whether such carriers can be the source of new outbreaks. A differential elisa test should ideally detect all (vaccinated) animals that are infected,

Recently, a diva strategy has been adopted to eliminate avian influenza virus from Italy. Flocks were vaccinated with a classical inactivated vaccine, containing the same haemagglutinin antigen as the outbreak virus but a different neuraminidase antigen. With a differential elisa that detects antibodies against the neuraminidase of the outbreak virus one could detect infected birds in vaccinated flocks. Because the combination of emergency diva vaccines and differential elisas is still not optimal, the decision as to whether or not to vaccinate in order to dampen down an outbreak is a difficult one: economical, social, scientific and ethical factors come into play. It may be expected that control strategies involving the destruction of millions of healthy animals will become gradually less acceptable.

Dr. Alberto Laddomada

FACTORS TO CONSIDER IN USING VACCINATION TO HELP CONTROL OUTBREAKS OF EXOTIC ANIMAL DISEASES

Dr. Ron DeHaven

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Use of vaccination is an important tool in disease control and can play a significant role in managing enzootic animal diseases or in eradication of disease outbreaks. Factors in addition to the efficacy of the vaccine need to be considered when electing to vaccinate. This is especially important for List A diseases or for diseases with trade implications in order to avoid real or perceived barriers to trade.

Many vaccines are highly effective in preventing disease symptoms. For enzootic diseases they may be used to control or even allow animal production when it would be either economically or scientifically difficult to maintain. When disease outbreaks occur, vaccines can be used to prevent clinical symptoms, to limit the spread of disease, or to assist in eradication efforts. When used in eradication or control programs, vaccines need to be evaluated for their ability to limit or eliminate agent shedding. Use of ring vaccination in the area adjacent to an outbreak is one example of an appropriate control measure when vaccines can limit or eliminate agent shedding.

Initiation of vaccination may limit trade when infected versus vaccinated animals cannot be differentiated. In such situations, vaccination is often a temporary measure, and vaccinated animals are intended to be removed from the population once disease control is obtained. Considerable planning and regulatory control is required to assure that animals are properly identified, appropriately contained, and promptly removed to avoid false positive results when surveillance measures are initiated.

When a disease crosses species, the effect of vaccination on trade needs to be considered even if only one species is intended to be vaccinated. Often the economic impact and loss of trade for the other species can outweigh the advantages of vaccination, and alternative control methods need to be considered.

Development of a decision-making process that considers the scientific, regulatory, economic, and industry concerns is important when considering vaccine use.

VACCINES AND FOOT-AND-MOUTH-DISEASE ERADICATION IN SOUTH AMERICA

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FMD vaccines have been a component of disease control and eradication strategies in South America, ever since the first national programs were created in the 60's. By mid 70's, with the aid of international loans, FMD control programs were implemented in almost every country and control measures strengthened. Livestock production forms are still a determining factor in the spread and prevalence of FMD and regional control/eradication strategies based on these forms were developed during the 80's, as part of the Hemispheric Plan for FMD Eradication, developed by Panafitosa-PAHO/WHO and the South American countries. The widespread use of oil-adjuvant vaccines and the development of strategic schemes of coverage were instrumental in decreasing clinical disease and in controlling FMD to a point that eradication could be sought. This resulted in the recognition of countries and regions as free with and without vaccination. Reappearance of FMD in Argentina, Southern Brazil and Uruguay were controlled with the aid of mass vaccination of bovines and other susceptible species, under special circumstances. Clinical FMD has been absent from Uruguay since August 2001; from Argentina since January 2002 and from Rio Grande do Sul, Brazil since July, 2001. To prevent reintroduction of FMDV into free areas, national programs must stress primary prevention activities with regional approaches and vaccination campaigns based on homogeneous coverage and timing, especially along international borders.