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A REVIEW

Application of marker vaccines against livestock diseases

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Effective control of any infectious disease in an endemic country needs better vaccines along with strong diagnostic support for the specific identification of the infecting agent. Traditional vaccine strategies using live attenuated and inactivated virus has been successful in the past for some of the diseases. But for the effective control of spreading infection during outbreak, along with the good immunization, the means of differentiating between the immunized animal and infected animal should be there so that immunization can be materialized in the proper way. Therefore, it is imperative that newer approaches to develop vaccines are explored at faster pace. The advent of newer methods in molecular biology and technical advances in DNA recombination has lead to the production of new innovative vaccine and subsequently to a new era in vaccinology.

A marker vaccine (live or inactivated vaccine) that can elicit a protective immunity distinguishable from the immune response elicited by the natural infection with the wild type virus. It is either based on deletion mutants or on isolated antigenic proteins that allows the distinction between vaccinated and infected animals on the basis of identifiable difference in antibody responses. A marker vaccine is used in conjunction with a companion diagnostic test that detects antibodies against a protein that is lacking in the vaccine strain. Animal diagnosed as positive for the presence of a field infection has to be eliminated regardless of prior vaccination with a marker vaccine for the effective control of disease. The term marker vaccine is misnomer because the cardinal feature is not that the antibody response of infected animals can be differentiated from that of vaccinated animals. Hence, DIVA (Differentiation of infected from vaccinated animals) vaccines have a negative marker because such vaccines carry at least one antigenic protein less than the corresponding wild-type virus.

Marker vaccines and companion diagnostic tests:

In animal health, one can either vaccinate animals in order to prevent a disease or try to eliminate the infection through strict application of sanitary measures such as slaughtering of infected and in-contact animals. Diagnosis of infection is of paramount importance whatever the measures taken to fight the disease. Diagnosis can be *direct*, through the detection and identification of the infectious agent using immunological or molecular technologies, or *indirect*, based upon the detection of specific antibodies against the suspected infectious agent. The latter methods have a major drawback in that one must wait until antibodies are synthesized by the animal after infection and generally they do not allow distinction between a humoral immune response resulting from an infection or a vaccination.This problem can be overcome by adopting new approaches to vaccine development using molecular technologies that allow the production of marker vaccines associated with companion diagnostic tests (eg.ELISA, PCR, etc.)

There are currently two types:

– based on the detection of a serological response against a protein whose gene has been deleted in the vaccine strain (either used as a replicating vaccine or as an inactivated vaccine derived from such a deleted virus

vaccine strain),

– based on the detection of the serological response to virus non-structural proteins (purified inactivated vaccines). In the case of the deletion vaccines the gene coding for a non-essential protein, the marker characteristic, is always linked with the detection test while in the case of subunit vaccines (e.g. protein E2 of classical swine fever virus expressed in baculovirus) the choice of the marker test assay may be linked to several other virus proteins. For harmonisation purposes, an agreed protein should be chosen for the test (e.g. protein gE of pseudorabies virus). In the first type of marker vaccines, the marker must always be negative since a positive marker, for instance provided through the insertion of a gene coding for a foreign protein, is not suitable; such a vaccine will only show if the animal has been vaccinated but will not indicate if the animal was also infected with the wild virus. Marker vaccine used with the intention of distinguishing a serological response resulting from either vaccination or infection must always be associated with a companion diagnostic test that can be used during a prophylactic campaign with the aim of eliminating the infectious agent.

Marker vaccines against viral and bacterial diseases:
Marker vaccines against viral diseases:

As there are no broad-spectrum antiviral pharmaceuticals available, hygienic measures to limit exposure and vaccination are the only means to prevent or control viral infections. Viruses (especially RNA viruses) are highly variable, and many viral infections are due to viruses with multiple serotypes (e.g., FMD virus, bluetongue virus, and influenza viruses). As a consequence, many of the existing viral vaccines are often unable to cope with the prevailing strains in the field, and new ones have to be generated from field strains with new outbreaks. Numerous conventional live and inactivated viral vaccines have been produced by animal health companies and have been used for many decades in routine vaccination protocols for both companion and production animals. Increasingly, a number of rationally designed marker vaccine (DIVA) and subunit vaccines are reaching the market. Such DIVA vaccines and their companion diagnostic tests are now available or in development for several diseases including infectious bovine rhinotracheitis (IBR), pseudorabies, classical swine fever (CSF), FMD, PRRS, NDV, Rinderpest, Bird flu, Equine Arteritis, etc. as detailed below.

Infectious Bovine Rhinotracheitis (IBR):

It is caused by *Bovine herpesvirus 1* (BHV-1) a virus of the family *Herpesviridae. Bovine herpesvirus type 1* (BHV-1) infection of cattles have been identified internationally as being candidates for eradication from national herds, and so there has been an impetus for the development of DIVA vaccines and diagnostics. The demand for a marker (DIVA) vaccine for IBR in Europe was met by the development of a glycoprotein E (gE)-deleted vaccine using conventional methodology(van Oirschot, *et al.,* 1996). The gE protein is not essential for viral replication, but it plays a major role in intercellular spread, particularly along nerves. Specific diagnostic tests based on gE deletion have been developed using both gE-blocking enzyme-linked immunosorbent assay (ELISA) techniques and PCR amplification (Perrin, *et al.,* 1996; Schynts *et al.,*1999).

Pseudorabies (Aujeszky's disease):

Pseudorabies is a viral disease in swine that is endemic in most parts of the world. It is caused by *porcine herpesvirus 1*, which is also called pseudorabies virus (PRV) or suid herpesvirus-1 (SHV-1) and is also known as Aujeszky's disease. Deletion of the gE gene has also been used to enable a DIVA approach for an Aujeszky's disease vaccine(Pensaert *et al.,* 2004). The gene for thymidine kinase is also deleted in some formulations(e.g., Suvaxyn Aujesky), adding to the degree of attenuation(Ferrari *et al.,* 2000). These deletion vaccines have been available since the 1980s, and their use has contributed to disease control and eradication in the United States and several European countries(Bouma, 2005).

Classical swine fever (CSF):

It is a highly contagious disease of swine caused by a small enveloped virus with a positive, single-stranded RNA genome, classified as a member of the genus *Pestivirus* within the family Flaviridae (Fauquet *et al*., 2005). Structural components of the CSFV virion include the capsid (C) protein and glycoproteins Erns, E1, and E2. E2 is the most immunogenic of the CSFV glycoproteins (Konig *et al*.,1995; van Gennip *et al*., 2000; Weiland *et al*., 1990), inducing neutralizing antibodies, which provide protection against lethal CSFV challenge. CSFV subunit marker vaccine produced in a baculovirus/insect cell system, formulated in a water-in-oil adjuvant, and accompanied by discriminatory ELISA tests are available (Hulst *et al*., 1993; van Rijn *et al*., 1996; 1999). The onset of immunity elicited by this subunit vaccines occurs 2 weeks post-vaccination, limiting their efficacy relative to traditional LAV vaccines when animals are exposed to CSFV shortly after vaccination (Bouma *et al*., 2000; Uttenthal *et al*., 2001). Infectious clone technology has

recently enabled antigenic modification of attenuated CSFV strains for use as experimental LAV marker vaccines. Infectious clones of the C-strain have been used to replace the antigenic region of E2 and/or the complete Erns gene with analogous sequences derived from Bovine Viral Diarrhea Virus (BVDV) (van Gianni *et al*., 2000). Also, promising experimental results were obtained with chimeric viruses using a BVDV infectious clone where the E2 gene was deleted and replaced by analogous sequences derived from CSFV strain Alfort (Reimann *et al*., 2004). Preliminary data suggest that all these chimeric vaccine viruses were able to induce protection in pigs and, significantly, chimera-induced anti-CSFV antibody responses could be discriminated from those produced by parental viruses.

Porcine reproductive and respiratory syndrome (PRRS):

PRRS is caused by arterivirus a small enveloped virus (PRRSV) containing a single, positive-stranded RNA genome that belongs to the family *Arteriviridae.* It was first isolated and classified as recently as 1991. The disease syndrome had been first recognised in the USA in the mid 1980's and was called "*mystery swine disease*". It has also been called *blue ear disease*. The name porcine arterivirus has been proposed recently. Here, a highly immunogenic epitope, ES4 gene located in the nsp2 region (aa 736–790 of pp1a) was deleted and replaced with the green fluorescent protein (GFP) gene (at aa 733/734 of pp1a) using reverse genetics. The resulting recombinant virus was characterized to determine its potential use as a marker vaccine against PRRSV infection. GFP antigen and ES4 peptide antigen-based ELISAs were tested to determine their sensitivity and specificity as companion diagnostic assays for marker detection and differentiating vaccinated animals from non-vaccinated ones (Fang, *et al*., 2008).

Rinderpest:

Rinderpest or cattle plague, is an economically important disease of domestic and wild ruminants. The disease is caused by rinderpest virus (RPV), which is classified in the genus *Morbillivirus* in the family *Paramyxoviridae*. The morbillivirus genome consists of a single strand of negative-sense RNA, which is organized into six contiguous, non-overlapping transcription units encoding six structural proteins, the nucleocapsid (N), polymerase (P), matrix (M), fusion (F), haemagglutinin (H) and large (L) proteins, in the order 3'-N-P-M-F-H-L-5' (Bailey *et al*., 2005; Crowley *et al*., 1988; Rima *et al*., 1986). The N protein is highly antigenic and most abundant of the structural proteins by virtue of its position close to the promoter (Cattaneo *et al*., 1987) and it performs several functions during virus replication. Therefore, it appears to be a suitable target for developing a marker vaccine for RPV. The N protein gene of the RPV virus strain was deleted and replaced with the equivalent gene from the PPRV virus. This recombinant virus exhibited growth characteristics in cell culture similar to those of the parental viruses, and animals vaccinated with this chimeric virus were protected from challenge with virulent virus. The C-terminal variable region of the N protein of morbilliviruses has been reported to protrude from the surface of the viral nucleocapsid (Heggeness *et al*., 1981) and is, therefore, a good candidate for developing a test for RPV/PPRV differential diagnosis. The C-terminal variable region of the RPV N protein when expressed in *Escherichia coli* could be used subsequently to develop an indirect ELISA for serological identification of animals vaccinated with the chimeric marker vaccine. The chimeric virus, when used in conjunction with the newly developed companion serological test, would be suitable for use in the final phase of the rinderpest eradication campaign.

Equine arteritis:

Caused by *Equine arteritis virus* (EAV) an enveloped plus-strand RNA virus of the family *Arteriviridae* (order *Nidovirales)* that causes respiratory and reproductive disease in equids which is a worldwide pathogen for horses and donkeys. The virus was first isolated from lung tissue of fetuses aborted during an outbreak in Ohio in 1953 (Doll *et al.,* 1957) and became the prototype arterivirus. The genome is packaged by the nucleocapsid protein (N) into an isometric nucleocapsid (Hyllseth, 1973; Zeegers *et al.,* 1976.) that is in turn surrounded by a lipid membrane. The envelope contains six proteins where G_L and M proteins are predominant (de Vries *et al.,* 1992). Protective, virus-neutralizing antibodies (VNAb) elicited by infection are directed predominantly against an immunodominant region in the membrane-proximal domain of the viral envelope glycoprotein G_L, allowing the establishment of a sensitive peptide enzyme-linked immunosorbent assay (ELISA) based on this particular domain (Nugent *et al*., J. Virol. Methods 90:167-183, 2000). The present study describes the generation of a EAV deletion mutant by deleting $G_{L\ gene}$ denoted by EAV-G_LD, which lacks amino acids (aa) 66 to 112 of the immunodominant domain of EAV G_L protein. This virus, EAV-G_LD, replicated to normal titers in culture cells, although at a slower rate than wild-type EAV, and caused an asymptomatic infection in ponies.

The antibodies induced neutralized the mutant virus efficiently in vitro but reacted poorly to wild-type EAV strains. Nevertheless, when inoculated subsequently with virulent EAV, the immunized animals, in contrast to non-vaccinated controls, were fully protected against disease; replication of the challenge virus occurred briefly at low though detectable levels. The levels of protection achieved suggest that an immune effector's mechanism other than VNAb plays an important role in protection against infection. As expected, infection with EAV-G_LD does not induce a measurable response in G_L-peptide ELISA while the challenge infection of the animals clearly does. EAV-G_LD or similar mutants are therefore attractive marker vaccine candidates, enabling serological discrimination between vaccinated and wild-type virus-infected animals.

FMD:

Foot-and-mouth disease, FMD or hoof-and-mouth disease (*Aphtae epizooticae*) is a highly contagious and sometimes fatal viral disease of cloven-hoofed animals which is caused by foot-and mouth-disease virus, a picornavirus, the prototypic member of the Aphthovirus genus in the picornaviridae family. There are seven FMD serotypes: O, A, C, SAT-1, SAT-2, SAT-3, and Asia-1. These serotypes show some regionality, and the O serotype is most common. Recent studies have identified a number of antigenic non-structural proteins (NSP) of FMD virus out of which *3ABC* gene appears to be the most reliable marker of FMD virus replication (Mackay *et al*., 1998, Sorensen *et al*., 1998). The deletion of NSP (3ABC) gene has been used for enabling DIVA approach for FMD (Cedivac-FMD inactivated vaccine). For detection of NSP antibodies, the Ceditest FMD-NS ELISA is commercially available. ELISA test for the detection of antibodies against non-structural proteins will play an essential role in the serological survey of livestock herds in future post-outbreak situations.

Bird flu (H5N1):

It is caused by *Influenza A virus* the genus of the *Orthomyxoviridae* family. H5N1 a subtype of Influenza A virus endemic to birds, currently perceived as a significant emerging pandemic threat. An interesting development in genetically engineered viral vaccine is the use of reverse genetic approach to construct the chimera vaccine (Poulvac FluFend i AI H5N3 RG) to protect poultry against the pathogenic H5N1 virus. Here the HA gene was deleted from an H5N1 virus (from a recent Asian outbreak), inactivated by removing the polybasic amino acid sequences, and combined with the NA gene from an H2N3 virus onto an H1N1 "backbone" virus. An immunoassay able to specifically detect antibodies against N3 and N1 proteins could be used for DIVA approach (*i.e.*, N3_ N1_ indicates vaccinated, and N3_ N1_ indicates infected). A further sophistication of this approach is a recently developed vaccine against avian influenza virus (Poulvac FluFend), where the hemagglutinin (HA) gene has been deleted from an H5N1 virus, inactivated by removing the polybasic amino acid sequences, and combined with the NA gene from an H2N3 virus onto an H1N1 "backbone" virus (Fig. 1). A vaccine containing the resultant inactivated H5N3-expressing virus administered in a water-in-oil emulsion protects chickens and ducks against the highly pathogenic H5N1 strain. (Li *et al*., 2008).

Newcastle disease:

Newcastle disease is a contagious bird disease which is caused by *Newcastle disease virus* (NDV) of

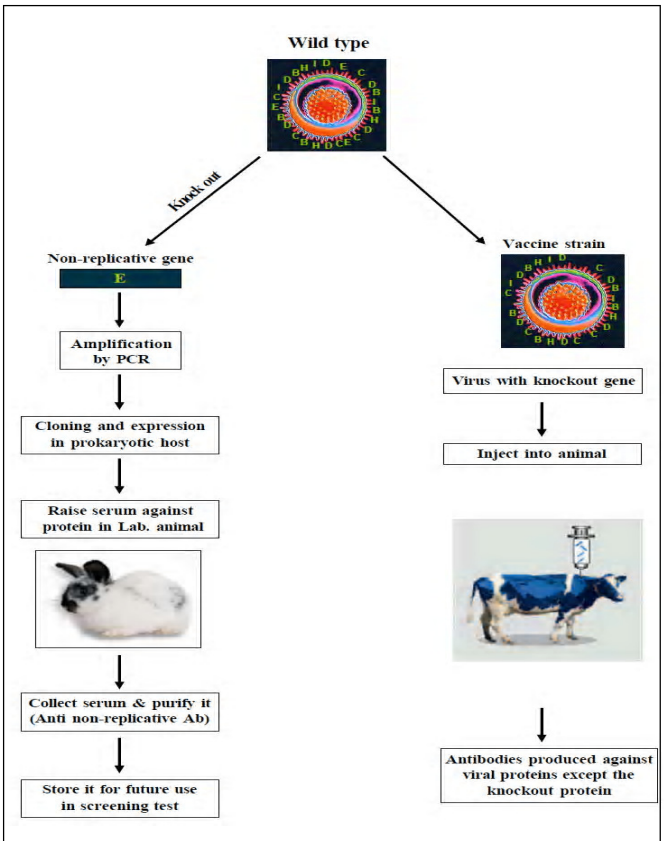

Fig. 1 : Schematic representation of marker vaccine preparation. The left side of the chart shows the knockout gene E which is amplified by PCR, cloned, expressed in prokaryotic cells, raising serum against protein and is purified. The right side shows the vaccine strain which lacks gene E that is used for vaccination

Genus *Avulavirus* and *paramyxoviridae* Family. It is a negative-sense single-stranded RNA virus. The nucleoprotein (NP) of Newcastle disease virus (NDV) functions primarily to encapsidate the virus genome for the purpose of RNA transcription, replication, and packaging. This conserved multifunctional protein is also efficient in inducing NDV-specific antibody in chickens. Here, the conserved B-cell immunodominant epitope NP-IDE gene was deleted and replaced by S2 glycoprotein of murine hepatitis virus (MHV). Chickens immunized with this hybrid recombinants virus produce specific antibodies against the S2 glycoprotein of MHV and completely lacked antibodies directed against the NP-IDE. These marked-NDV recombinants, in conjunction with a diagnostic test, enable serological differentiation of vaccinated animals from infected animals and may be useful tools in ND eradication programs (Mebatsion *et al*., 2002). The identification of a mutation-permissive region on the NP gene allows a rational approach to the insertion of protective epitopes and may be relevant for the design of NDV-based cross-protective marker vaccines.

Marker vaccines against Bacterial diseases:

In case of bacterial dieseases live vaccines has been prepared by multiple passages in various media in the hope that some random mutation would deliver a nonvirulent and attenuation of bacteria, but at the same time it remain replicable. With currently used molecular methods, the obtained deletions/mutations can be identified, but this technology also allows a more targeted design of live vaccines with specific deletions of predetermined known genes. Good targets for these deletions are genes responsible for key metabolic processes that inhibit the spread of the infection but allow the development of immune responses against virulence factors. Alternatively, deletions of virulence-associated genes are targets, but this may be more problematic when a protective immune response is desired.

Strangles:

A highly contagious disease in horses caused by infection with *Streptococcus equi* subsp. *equi*. Recently, Gene-deleted vaccines (Equilis StrepE vaccine) against strangles was licensed in Europe. This is a live recombinant vaccine prepared from the *S. equi* TW928 deletion mutant lacking bp 46 to 978 of the *aroA* gene (Jacobs *et al.,* 2000; Kelly *et al.,* 2006). This mutant was constructed by the electroporation of gene knockout and gene deletion constructs. No foreign DNA such as antibiotic resistance markers was introduced, but the vaccine strain can allegedly be identified by an *aroA* PCR identifying the partial gene deletion (Kelly *et al.,* 2006). The live gene-deleted attenuated vaccine strain was originally developed for intranasal application, but protection was accomplished only by intramuscular injections, which in turn resulted in the local swelling of muscle tissue and the eventual formation of abscesses at the vaccination site (Jacobs *et al.,* 2000). However, sub mucosal administration of the vaccine in the upper lip was shown to confer protection comparable to that of intramuscular administration with only minimal local reactions (Jacobs *et al.,* 2000), and it is with this unusual route of administration that the vaccine is now licensed.

Porcine pleuropneumonia:

Actinobacillus pleuropneumoniae, the cause of porcine pleuropneumonia,is a major economic problem in the swine industry worldwide (Fenwick and Henry, 1994). Deletions were introduced into the *ureC* and *apxIIA* genes of an *Actinobacillus pleuropneumoniae* serotype 2 strains by homologous recombination and counter selection. The double gene deletion is highly attenuated, protects pigs from homologous challenge upon a single aerosol application, and facilitates the serological discrimination of immunized and infected herds. The *apxIIA* gene was deleted as it encodes a highly immunogenic virulence factor expressed by all *A. pleuropneumoniae* serotypes except serotype 10; it has been used for serodiagnosis (Belli *et al.,* 2004) and, therefore, could be used for discrimination of immunized

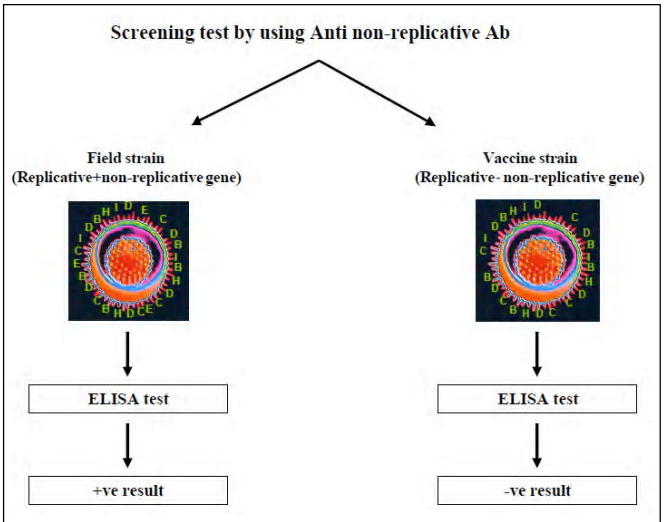

Fig. 2 : Schemetic representation showing the differential diagnosis using anti-non-replicative antibody. The left side (field strain) shows positive result in ELISA test. The right side (vaccine strain) it shows negative result

and infected herds in routine diagnostics. The *ureC* gene was deleted in order to potentially reduce shedding of the vaccine strain (Allan, G. M., *et al.,* 2004); in addition, it can serve as a reliable phenotypic marker to discriminate between the vaccine and the wild-type strain.

Salmonellosis:

It is caused by *Salmonella sp*. For DIVA approach to this disease a double-gene- (Aro gene) was deleted from *Salmonella enterica* serovar Typhimurium. The birds vaccinated with this live attenuated marker vaccine (MeganVac 1) shows protection against the wild type. ELISA test is used as a companion diagnostic assay for marker detection and differentiating vaccinated birds from non-vaccinated ones. The MeganVac 1 organism has also been reformulated for immunization of laying hens (MeganEgg). The Megan vaccines for broilers and hens were licensed by the USDA in 1998 and 2003, respectively (Tonpitak *et al*., 2002).

Conclusions:

Now a days there are new type of vaccine invented known as Subunit vaccine based on the important viral protein or glycoprotein produced in special expression system and formulated with appropiate adjuvent accompanied by discriminatory ELISA tests. Subunit antigen approaches to vaccination have been largely ineffective, as they present only a limited number of epitopes to the animal's immune system, and multiple antigens are generally required for protection. Though these vaccines will allow a DIVA approach to emergency vaccination and disease control in the case of new outbreaks, these have not yet been used widely in the field and appear to be less protective than conventional live, attenuated viral vaccines. Synthetic peptide vaccines have also been produced, however, so far they have not been shown to be very effective in inducing protection against infectious diseases. Current research is focused largely on combinations of capsid proteins, including empty capsid delivered by various expression systems, and the development of sensitive tests (ELISA) for antibodies against nonstructural proteins. Though we have seen so many advances and development in vaccinology during the past decades, there is still lots yet to be achieved.

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REFERENCES

Alexandersen, S., Quan, M., Murphy, C., Knight, J. and Zhang, Z. (2003). Studies of Quantitative Parameters of Virus Excretion and Transmission in Pigs and Cattle Experimentally Infected with Foot-and-Mouth Disease *Virus. J. Comp. Path.,* **129** (4): pp. 268-282.

Allan, G. M., McNeilly, F., Ellis, J., Krakowka, S., Botner, A., McCullough, K., Nauwynck, H., Kennedy, S., Meehan, B. and Charreyre, C. (2004). PMWS: experimental model and co-infections. *Vet. Microbiol.,* **98**:165–168.

Babu, U., Dalloul, R. A., Okamura, M., Lillehoj, H. S., Xie, H., Raybourne, R. B., Gaines, D. and Heckert, R. A. (2004). Salmonella enteritidis clearance and immune responses in chickens following Salmonella vaccination and challenge. *Vet. Immunol. Immunopathol.,* **101** : 251–257.

Bailey, D., Banyard, A., Dash, P., Ozkul, A. and Barrett, T. (2005). Full genome sequence of peste des petits ruminants virus, a member of the Morbillivirus genus. *Virus Res.,* **110** : 119–124.

Balamurugan, V., Sen, A., Saravanan P. and Singh, R.K. (2006). Biotechnology in the Production of Recombinant Vaccine of antigen for Animal Health. *J. Animal & Veterinary Advances*, **5**(6): 487-495.

Baltes, N., Hennig-Pauka, I. and Gerlach, G.F. (2002). Both transferring binding proteins are virulence factors in Actinobacillus pleuropneumoniae serotype 7 infection. *FEMS Microbiol. Lett.,* **209**:283–287.

Baltes, N., Tonpitak, W., Gerlach, G. F., Hennig-Pauka, I., Hoffmann Moujahid, A., Ganter, M. and Rothkotter, H.J. (2001). Actinobacillus pleuropneumoniae iron transport and urease activity: effects on bacterial virulence and host immune response. *Infect. Immun.,* **69**:472–478.

Beer, M., Reimann, I., Hoffmann, B. and Depner, K. (2007). Novel marker vaccines against classical swine fever virus. *Vaccine,* **25** : 5665–5670.

Belli, S. I., Mai, K., Skene, C.D., Gleeson, M.T., Witcombe, D.M., Katrib, M., Finger, A., Wallach, M.G. and Smith, N.C. (2004). Characterisation of the antigenic and immunogenic properties of bacterially expressed, sexual stage antigens of the coccidian parasite, Eimeria maxima. *Vaccine,* **22** : 4316–4325.

Bouma, A. (2005). Determination of the effectiveness of Pseudorabies marker vaccines in experiments and field trials. *Biologicals,* **33** : 241–245.

Brown, D. D., Collins, F. M., Duprex, W. P., Baron, M. D., Barrett, T. and Rima, B. K. (2005). Rescue' of mini-genomic constructs and viruses by combinations of morbillivirus N, P and L proteins. *J. Gen. Virol.,* **86** : 1077–1081.

Castillo-Olivares, J., de Vries, A.A.F., Raamsman, M.J.B., Rottier, P.J.M., Lakhani, K., Westcott, D., Tearle, J.P., Wood, J.L.N., Mumford, J.A., Hannant, D. and Davis-Poynter, N.J. (2001). Evaluation of a prototype sub-unit vaccine against

equine arteritis virus comprising the entire ectodomain of the virus large envelope glycoprotein (GL): induction of virus-neutralizing antibody and assessment of protection in ponies. *J. Gen. Virol.,* **82**:2425-2435.

Chang, C.F., Yeh, T. M., Chou, C.C., Chang, Y. F. and Chiang, T. S. (2002). Antimicrobial susceptibility and plasmid analysis of Actinobacillus pleuropneumoniae isolated in Taiwan. *Vet. Microbiol.,* **84** : 169–177.

Chen, H., Deng, G., Li, Z., Tian, G. , Li, Y., Jiao, P., Zhang, L., Liu, Z., Webster, R.G. and Yu, K. (2004). The evolution of H5N1 influenza viruses in ducks in southern China, *Proc. Natl. Acad. Sci. USA,* **101** : 10452–10457.

Chen, H., Li, Y., Li, Z., Shi, J., Shinya, K, Deng, G. , Qi, Q., Tian, G., Fan, S., Zhao, H., Sun, Y. and Kawaoka, Y. (2006). Properties and dissemination of H5N1 viruses isolated during an influenza outbreak in migratory waterfowl in western China, *J. Virol.,* **80** : 5976–5983.

Dechamma, H. J., Dighe, V., Kumar, C. A., Singh, R. P., Jagadish, M. and Kumar, S. (2006). Identification of T-helper and linear B epitope in the hypervariable region of nucleocapsid protein of PPRV and its use in the development of specific antibodies to detect viral antigen. *Vet. Microbiol.,* **118** : 201–211

de Lima, M., Kwon, B., Ansari, I. H., Pattnaik, A. K., Flores, E. F. and Osorio, F. A. (2008). Development of a porcine reproductive and respiratory syndrome virus differentiable (DIVA) strain through deletion of specific immunodominant epitopes. *Vaccine,* **26** : 3594–3600.

De Simone, F., Yadin, H., Haas, B., Bulut, N., Malirat, V., Neitzert, E., Goris, N., Parida, S.,Sørensen, K. and De Clercq, K. 2006. Comparative performance of six ELISAs for antibodies to thenon-structural proteins of foot-and-mouth disease. Vaccine (in press).

de Vries, A.A.F., Chirnside, E.D., Horzinek, M.C. and Rottier, P.J.M. (1992). Structural proteins of equine arteritis virus. *J. Virol.,* **66** : 6294-6303.

Doll, E.R., Bryans, J.T., McCollum, W.H. and Crowe, M.E.W. (1957). Isolation of a filterable agent causing arteritis of horses and abortion by mares. Its differentiation from the equine abortion (influenza) virus. *Cornell Vet.,* **47**:3-41. 26

Doll, E.R., Knappenberg, R.E. and Bryans, J.T. (1957). An outbreak of abortion caused by the equine arteritis virus. *Cornell Vet.,* **47** : 69-75.

Epizooties, O.I.E. (2004). OIE manual of diagnostic tests and vaccines for terrestrial animals, Office International des Epizooties, Paris, France.

Fang, Y., Rowland, R. R. R., Roof, M., Lunney, J. K., Christopher-Hennings, J. and Nelson, E. A. (2006). A full-length cDNA infectious clone of North American type 1 porcine reproductive and respiratory syndrome virus: expression of green fluorescent protein in the Nsp2 region. *J. Virol.,* **80** : 11447–11455.

Fenwick, B. and Henry, S. (1994). Porcine pleuropneumonia. *J. Am. Vet. Med. Assoc.,* **204** : 1334–1340.

Ferrari, M., Brack, A., Romanelli, M.G., Mettenleiter, T.C., Corradi, A., Dal Mas, N., Losio, M. N., Silini, R., Pinoni, C. and Pratelli, A. (2000). A study of the ability of a TK-negative and gI/gE-negative pseudorabies virus (PRV) mutant inoculated by different routes to protect pigs against PRV infection. *J. Vet. Med. B Infect. Dis. Vet. Public Health,* **47**:753–762.

Gerdts, V., Mutwiri, G. K., Tikoo, S. K. and Babiuk, L.A. (2006). Mucosal delivery of vaccines in domestic animals. *Vet. Res.,* **37** : 487–510.

Gerhardt, R. (2006). West Nile virus in the United States (1999–2005). *J Am. Anim. Hosp. Assoc.,* **42**:170–177.

Ghosh, S., Walker, J. and Jackson, D.C. (2001). Identification of canine helper T-cell epitopes from the fusion protein of canine distemper virus. *Immunology,* **104** : 58–66.

Govindarajan, D., Buchholz, U.J. and Samal, S. K. (2006). Recovery of avian metapneumovirus subgroup C from cDNA: cross-recognition of avian and human metapneumovirus support proteins. *J. Virol.,* **80** : 5790–5797.

Gould, C. (2003). Successful oral vaccination. *Skretting Outlook,* **20** : 10–11.

Graham, S. P., Pelle, R., Honda, Y., Mwangi, D.M., Tonukari, N.J., Yamage, M., Glew, E. J., de Villiers, E. P., Shah, T., Bishop, R., Abuya, E., Awino, E., Gachanja, J., Luyai, A. E., Mbwika, F., Muthiani, A. M., Ndegwa, D. M. , Njahira, M., Nyanjui, J. K., Onono, F. O. , Osaso, J., Saya, R. M., Wildmann, C., Fraser, C. M., Maudlin, I., Gardner, M. J., Morzaria, S.P., Loosmore, S., Gilbert, S.C. , Audonnet, J.C. , van der Bruggen, P., Nene, V. and Taracha, E.L. (2006). Theileria parva candidate vaccine antigens recognized by immune bovine cytotoxic T lymphocytes. *Proc. Natl. Acad. Sci. USA,* **103**:3286–3291.

Grubman, M. J. (2005). Development of novel strategies to control foot-andmouth disease: marker vaccines and antivirals. *Biologicals,* **33**:227–234.

Gubbins, S. and Parida, S. (2006). Application of non-structural protein antibody tests in substantiating freedom from foot-and-mouth disease virus infection after emergency vaccination of cattle Vaccine.

Guedes, R. M. and Gebhart, C.J. (2003). Onset and duration of fecal shedding, cell-mediated and humoral immune responses in pigs after challenge with a pathogenic isolate or attenuated vaccine strain of Lawsonia intracellularis. *Vet. Microbiol.,* **91**:135–145.

Haesebrouck, F., Pasmans, F., Chiers, K., Maes, D., Ducatelle, R. and Decostere, A. (2004). Efficacy of vaccines against bacterial diseases in swine: what can we expect? *Vet. Microbiol.,* **100**:255–268.

Han, J., Liu, G., Wang, Y. and Faaberg, K.S. (2007). Identification of nonessential regions of the nsp2 replicase protein of porcine reproductive and respiratory syndrome virus strain VR-2332 for replication in cell culture. *J. Virol.,* **81** : 9878–9890.

Hardham, J., Dreier, K., Wong, J., Sfintescu, C. and Evans, R.T. (2005). Pigmented-anaerobic bacteria associated with canine periodontitis. *Vet. Microbiol.,* **106**:119–128.

Hardham, J., Reed, M., Wong, J., King, K., Laurinat, B., Sfintescu, C. and Evans, R.T. (2005). Evaluation of a monovalent companion animal periodontal disease vaccine in an experimental mouse periodontitis model. *Vaccine,* **23**:3148–3156.

Hayes, E.B. and Gubler, D.J. (2006). West Nile virus: epidemiology and clinical features of an emerging epidemic in the United States. *Annu. Rev. Med.,* **57** : 181–194.

Heppell, J. and Davis, H.L. (2000). Application of DNA vaccine technology to aquaculture. *Adv. Drug Deliv. Rev.,* **43**:29–43.

Hogge, G.S., Burkholder, J.K., Culp, J., Albertini, M.R., Dubielzig, R.R., Yang, N.S. and MacEwen, E.G. (1999). Preclinical development of human granulocyte-macrophage colony-stimulating factor-transfected melanoma cell vaccine using established canine cell lines and normal dogs. *Cancer Gene Ther.,* **6**:26–36.

Hoskinson, R.M., Rigby, R.D., Mattner, P.E., Huynh, V.L., D'Occhio, M. , Neish, A., Trigg, T.E., Moss, B.A., Lindsey, M.J., Coleman, G. D. (1990). Vaxstrate: an anti-reproductive vaccine for cattle. *Australian J. Biotechnol.,* **4**:166–170, 176.

Huleatt, J.W., Jacobs, A.R., Tang, J., Desai, P., Kopp, E.B., Huang, Y., Song, L., Nakaar, V. and Powell, T.J. (2007). Vaccination with recombinant fusion proteins incorporating Toll-like receptor ligands induces rapid cellular and humoral immunity. *Vaccine,* **25** : 763–775.

Hyllseth, B. (1973). Structural proteins of equine arteritis virus. Arch. Gesamte *Virusforsch.*, **40** : 177-188. 39 4032.

Innes, E.A., Wright, S., Bartley, P., Maley, S., Macaldowie, C., Esteban Redondo, I. and Buxton, D. (2005). The host-parasite relationship in bovine neosporosis. *Vet. Immunol. Immunopathol.,* **108** : 29–36.

Jackwood, M.W. and Saif, Y.M. (1985). Efficacy of a commercial turkey coryza vaccine (Art-Vax) in turkey poults. *Avian Dis.,* **29**:1130–1139.

Jacobs, A.A., Goovaerts, D., Nuijten, P.J., Theelen, R.P., Hartford, O.M. and Foster, T.J. (2000). Investigations towards an efficacious and safe strangles vaccine: submucosal vaccination with a live attenuated Streptococcus equi. *Vet. Rec.,* **147**:563–567.

Jacobs, A.A., Goovaerts, D., Nuijten, P.J., Theelen, R.P., Hartford,O.M. and Foster, T.J. (2000). Investigations towards an efficacious and safe strangles vaccine: submucosal vaccination with a live attenuated Streptococcus equi. *Vet. Rec.,* **147** : 563–567.

Jarosinski, K.W., Tischer, B.K., Trapp, S. and Osterrieder, N. (2006). Marek's disease virus: lytic replication, oncogenesis and control. *Expert Rev. Vaccines,* **5** : 761–772.

Johnson, K.S., Harrison, G.B., Lightowlers, M.W., O'Hoy, K.L., Cougle, W.G., Dempster, R.P., Lawrence, S.B., Vinton, J.G. , Heath, D.D. and Rickard, M.D. (1989). Vaccination against ovine cysticercosis using a defined recombinant antigen. *Nature,* **338** : 585–587.

Kahn, L.H. (2006). Confronting zoonoses, linking human and veterinary medicine. *Emerg. Infect. Dis.,* **12** : 556–561.

Kelly, C., Bugg, M., Robinson, C., Mitchell, Z., Davis-Poynter, N., Newton, J.R., Jolley, K.A., Maiden, M.C. and Waller, A.S. (2006). Sequence variation of the SeM gene of Streptococcus equi allows discrimination of the source of strangles outbreaks. *J. Clin. Microbiol.,* **44** : 480–486.

Kennedy, N.J., Spithill, T.W., Tennent, J., Wood, P.R. and Piedrafita, D. (2006). DNA vaccines in sheep: CTLA-4 mediated targeting and CpG motifs enhance immunogenicity in a DNA prime/protein boost strategy. *Vaccine,* **24** : 970–979.

Killian, G., Miller, L., Rhyan, J. and Doten, H. (2006). Immunocontraception of Florida feral swine with a single-dose GnRH vaccine. *Am. J. Reprod. Immunol.,* **55** : 378–384.

Kim, D. Y., Calvert, J. G., Chang, K. O., Horlen, K., Kerrigan, M. and Rowland, R. R. R. (2007). Expression and stability of foreign tags inserted into nsp2 of porcine reproductive and respiratory syndrome virus (PRRSV). *Virus Res.,* **128** : 106–114.

Kirkbride, C.A. (1993). Diagnoses in 1,784 ovine abortions and stillbirths. *J. Vet. Diagn. Investig.,* **5** : 398–402.

Kocan, K.M., de la Fuente, J., Guglielmone, A.A. and Melendez, R.D. (2003). Antigens and alternatives for control of Anaplasma marginale infection in cattle. *Clin. Microbiol. Rev.,* **16** : 698–712.

Ko¨ nig, P., Hoffmann, B., Depner, K.R., Reimann, I., Teifke, J.P. and Beer, M. (2007a). Detection of classical swine fever vaccine virus in blood and tissue samples of pigs vaccinated either with a conventional C-strain vaccine or a modified live marker vaccine. *Vet. Microbiol.,* **120** : 343–351.

Ko¨ nig, P., Lange, E., Reimann, I. and Beer, M. (2007b). CP7_E2alf: a safe and efficient marker vaccine strain for oral immunisation of wild boar against classical swine fever virus (CSFV). *Vaccine,* **25** : 3391–3399.

Kreeger, T.J., Cook, W.E., Edwards, W.H., Elzer, P.H. and Olsen, S.C. (2002). Brucella abortus strain RB51 vaccination in elk. II. Failure of high dosage to prevent abortion. *J. Wildl. Dis.,* **38** : 27–31.

Kroll, J.J., Roof, M.B. and McOrist, S. (2004). Evaluation of protective immunity in pigs following oral administration of an avirulent live vaccine of Lawsonia intracellularis. *Am. J. Vet. Res.,* **65** : 559–565.

Kuiken, T., Rimmelzwaan, G. , van Riel, D., van Amerongen, G., Baars, M., Fouchier, R. and Osterhaus, A. (2004). Avian H5N1 influenza in cats. *Sci.,* **306** : 241.

Ladd, A.E., Wang, C.Y. and Zamb, T.J. (1998). Immunogenic LHRH peptide constructs and synthetic universal immune stimulators for vaccines. U.S. patent, 5843446.

Lambert, P.H., Liu, M. and Siegrist, C.A. (2005). Can successful vaccines teach us how to induce efficient protective immune responses? *Nat. Med.,* **11**: S54–S62.

Larche, M., Akdis, C.A. and Valenta, R. (2006). Immunological mechanisms of allergen-specific immunotherapy. *Nat. Rev. Immunol.,* **6** : 761–771.

Lea, I.A., Widgren, E.E. and O'Rand, M.G. (2002). Analysis of recombinant mouse zona pellucida protein 2 (ZP2) constructs for immunocontraception. *Vaccine*, **20** : 1515–1523.

Lehman, J.R., Weigel, R.M., Siegel, A.M., Herr, L.G., Taft, A.C. and Hall, W.F. (1993). Progress after one year of a pseudorabies eradication program for large swine herds. *J. Am. Vet. Med. Assoc.,* **203**:118–121.

Liang, R., van den Hurk, J.V., Babiuk, L.A. and van Drunen Littel-van den Hurk, S. (2006). Priming with DNA encoding E2 and boosting with E2 protein formulated with CpG oligodeoxynucleotides induces strong immune responses and protection from bovine viral diarrhea virus in cattle. *J. Gen. Virol.,* **87**:2971–2982.

Liu, S., Tu, C., Wang, C., Yu, X., Wu, J., Guo, S., Shao, M., Gong, Q., Zhu, Q. and Kong, X. (2006). The protective immune response induced by B cell epitope of classical swine fever virus glycoprotein E2. *J. Virol. Methods,* **134** : 125–129.

Li, Z., Chen, H., Jiao, P., Deng, G., Tian, G., Li, Y., Hoffmann, E., Webster, R.G., Matsuoka, Y. and Yu, K. (2005). Molecular basis of replication of duck H5N1 influenza viruses in a mammalian mouse model, *J. Virol.,* **79** : 12058–12064.

Mahapatra, M., Parida, S., Baron, M.D. and Barrett, T. (2006). Matrix protein and glycoproteins F and H of peste-des-petits-ruminants virus function better as a homologous complex. *J. Gen. Virol.,* **87** : 2021–2029.

Martelli, P., Cordioli, P., Alborali, L. G., Gozio, S., De Angelis, E., Ferrari, L., Lombardi, G. and Borghetti, P. (2007). Protection and immune response in pigs intradermally vaccinated against porcine reproductive and respiratory syndrome (PRRS) and subsequently exposed to a heterologous European (Italian cluster) field strain. *Vaccine,* **25** : 3400–3408.

McCollum, W. H. (2002). Studies of passive immunity in foals to equine viral arteritis. *Vet. Microbiol.,* **1**:45-54

Mebatsion, T., Verstegen, S., de Vaan, L.T.C. Römer-Oberdörfer, A. and Schrier, C. (2001). A recombinant Newcastle disease virus with low-level V protein expression is immunogenic and lacks pathogenicity for chicken embryos. *J. Virol.,* **75** : 420-428

Meeusen, E.N.T., Walker, J., Peters, A., Pastoret, P. and Jungersen, G. (2007). Current Status of Veterinary Vaccines. *Clinical Microbiology Reviews.* **20** (3): 489–510.

Neumann, E. J., Kliebenstein, J.B., Johnson, C. D., Mabry, J. W., Bush, E. J., Seitzinger, A. H., Green, A. L. and Zimmerman, J. J. (2005). Assessment of the economic impact of porcine reproductive and respiratory syndrome on swine production in the United States. *J. Am. Vet. Med. Assoc.,* **227** : 385–392.

Nugent, J., Sinclair, R., de Vries, A.A.F., Eberhardt, R.Y., Castillo-Olivares, J., Davis-Poynter, N.J., Rottier, P.J.M. and Mumford, J.A. (2000). Development and evaluation of ELISA procedures to detect antibodies against the major envelope protein (G(L)) of equine arteritis virus. *J. Virol. Methods,* **90** : 167-183.

Orsel, K., Dekker, A., Bouma, A., Stegeman, J.A. and Jong, M.C. (2005). Vaccination against foot and mouth disease reduces virus transmission in groups of calves, *Vaccine,* **23** (41): 4887–4894.

Parida, S., Mahapatra, M., Hawes, P., Baron, M. D., Monaghan, P. and Barrett, T. (2006). Importance of the extracellular and cytoplasmic/transmembrane domains of the haemagglutinin protein of rinderpest virus for recovery of viable virus from cDNA copies. *Virus Res.,* **117** : 273–282.

Panel of recombinant, non-structural proteins in ELISA. *Vaccine,* **16** (5): 446-459.

Peeters, B.P.H., De Leeuw, O.S., Verstegen, I., Koch, G. and Gielkens, A.L.J. (2001). Generation of a recombinant chimeric Newcastle disease virus vaccine that allows serological differentiation between vaccinated and infected animals. *Vaccine,* **19**:1616-1627

Pensaert, M., G. Labarque, H. Favoreel, and H. Nauwynck. 2004. Aujeszky's disease vaccination and differentiation of vaccinated from infected pigs. *Dev. Biol. (Basel),* **119** : 243–254.

Perrin, B., Perrin, M., Moussa, A. and Coudert, M. (1996). Evaluation of a commercial gE blocking ELISA test for detection of antibodies to infectious bovine rhinotracheitis virus. *Vet. Rec.,* 138:520.

Ran, Z. G., Chen, X. Y., Guo, X., Ge, X. N., Yoon, K. J. and Yang, H. C. (2008). Recovery of viable porcine reproductive and respiratory syndrome virus from an infectious clone containing a partial deletion within the Nsp2-encoding region. *Arch. Virol.,* **153** : 899–907.

Rasmussen, T.B., Uttenthal, A., Reimann, I., Nielsen, J., Depner, K. and Beer, M. (2007). Virulence, immunogenicity and vaccine properties of a novel chimeric pestivirus. *J. Gen. Virol.,* **88** : 481–486.

Reimann, I., Semmler, I. and Beer, M. (2007). Packaged replicons of bovine viral diarrhea virus are capable of inducing a protective immune response. *Virology,* **366** : 377–386.

Reynolds, D.L. and Maraqa, A.D. (2000). Protective immunity against Newcastle disease: the role of cell-mediated immunity. *Avian Dis.,* **44** : 145-154.

Schynts, F., Baranowski, E., Lemaire, M. and Thiry, E. (1999). A specific PCR to differentiate between gE negative vaccine and wildtype bovine herpesvirus type 1 strains. *Vet. Microbiol.,* **66**:187–195.

Tian, G. , Zhang, S., Li, Y., Bu, Z., Liu, P., Zhou, J., Li, C., Shi, J., Yu, K. and Chen, H. (2005). Protective efficacy in chickens, geese and ducks of an H5N1-inactivated vaccine developed by reverse genetics, *Virology,* **341** :153–162.

Vannier, P., Capua, I., Le Potier, M.F., Mackay, D.K.J., Muylkens, B., Parida, S., Paton, D.J. and Thiry, E. (2007). Marker vaccines and the impact of their use on diagnosis and prophylactic measures. *Rev. Sci. Tech. Off. Int. Epiz.,* **26** (2) : 351-372.

van Oirschot, J.T., Kaashoek, M.J. and Rijsewijk, F.A. (1996). Advances in the development and evaluation of bovine herpesvirus 1 vaccines. *Vet. Microbiol.,* **53** : 43–54.

Ward, M.D.W., Frederick, J., Mehrotra, Y. and De Buysscher, E.V. (2000). Nucleotide sequence and vaccinia expression of the nucleoprotein of a highly virulent, neurotropic strain of Newcastle disease virus. *Avian Dis.,* **44** : 34-44

Weigel, R., **Hahn, E.C.** and Scherba, G. (2003). Survival and immunization of raccoons after exposure to pseudorabies (Aujeszky's disease) virus gene-deleted vaccines. *Vet. Microbiol*., **92** : 19-24.

Zeegers, J.J.W., van der Zeijst, B.A.M. and Horzinek, M.C. (1976). The structural proteins of equine arteritis virus. *Virology,* **73**:200-205.

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