epidemic regions. Using this dataset we investigated genetic diversity, patterns of distribution, and evolutionary history. **Results**: Our analysis indicates that the rabies virus in China is primarily defined by two clades that exhibit distinct population subdivision and translocation patterns and that contributed to the epidemic in different ways. The younger clade originated around 1992 and has properties that closely match the observed spread of the recent epidemic. The older clade originated around 1960 and has a dispersion pattern that suggests it represents a strain associated with a previous outbreak that remained at low levels throughout the country and reemerged in the current epidemic. **Conclusions**: Our findings provide new insight into factors associated with the recent epidemic and are relevant to determining an effective policy for controlling the virus.

CO.44
PLAYING THE ODDS: PRIORITIZING HUMAN RABIES BIOLOGICS IN LIMITED SUPPLY SCENARIOS

Recuenco S[1], Vora NM[1], Rupprecht C[1] – [1]CDC – Rabies Program

Limitations in the availability and access to human rabies biologics in enzootic regions result in most rabies deaths in the developing world. Efforts to supply modern rabies vaccines and immune globulin (RIG) have improved availability, but cost and the lack of structured programs in many countries remain major obstacles to providing optimal care. Proposed policies to provide rabies post-exposure prophylaxis (PEP) at no cost to the patient through government programs are challenged by the limited supply of rabies biologics that providers are able to obtain. In many cases, the demand for biologics exceeds the limited supplies and national rabies programs are therefore forced to ration, resulting in delays or complete failures in provision of adequate PEP. Optimal PEP involves the use of rabies immune globulin and vaccine. While WHO recommendations for PEP are comprehensive, those recommendations offer no guidance on management of rabies exposures when there are limited supplies of biologics in the country nor if there is only vaccine available but no RIG. Complex operationalization issues, such as to how to approach prioritization when both nervous tissue and modern vaccines coexist in a country, or how to optimally integrate private distribution of rabies biologics, are not part of the WHO guidance documents. We present a proposal on how to develop recommendations and guidelines to deal with these scenarios accounting for local rabies epidemiology, patient age and body size, delays after exposure, and cultural and social issues. Several Old and New World country cases are presented to highlight how these challenging circumstances might be managed and overcome.

CO.45
IMMUNE RESPONSE OF BALB/C MICE IMMUNIZED WITH VERO CELL RABIES VACCINE AND BpMPLA-SE ADJUVANT

Frazatti-Gallina NM[1], Silva ABP, Rinaldi DP, Silveri A, Raw I, Menezes CRB – [1]Instituto Butantan – Laboratório de Raiva

The prophylaxis is an important strategy to control of human and animal rabies disease. The vaccine from Vero cellular culture for human use is efficacy and safety. However, because the technology used to produce this vaccine is expensive this product costs about ten dollars. This cost makes them impossible the use of this vaccine type in poor countries where the animal rabies control is inefficient and there many cases of human rabies. Rabies disease is responsible for about 55,000 deaths per year in the world. The objective of this study was evaluate the humoral immune response of mice (Balb/c) immunized with three different doses of Vero rabies vaccine associated with the BpMPLA-SE adjuvant. This adjuvant is a product obtained from Bordetella pertusis. Three groups of ten mice were immunized with two doses of 500µl (G1), 250µl (G2) or 125µl (G3) of Vero cell rabies vaccine (IB-lot 1103075) mixed with BpMPLA-SE (10µg/dose). Three groups control (Gc) received only rabies vaccine. The immunization occurred on days 0 and 21 and samples were taken ten days after the last dose injected and on days 60, 120 and 180 to determine the titers of neutralizing antibodies for rabies virus in BHK21 cells (RFFIT). The averages of the neutralizing antibodies titers found in the samples from each group ten days after finished the immunization were 39.2, 33.1, and 20.4 IU/ml for groups G1, G2 and G3 respectively. The results obtained on day 180 were 17.1 IU /ml (G1), 10.6 IU/ml (G2) and 9.8 (G3). In the control groups the averages of the antibodies titers were: 29.7 (Gc1), 26.9 (Gc2) and 22.2 IU/ml (Gc3) after immunization and 10.7 (Gc1), 9.5 (Gc2) and 8.5 IU/ml (GC3) on day 180 (Gc3). These data show that the adjuvant BpMPLA-SE increased the humoral immune response for rabies vaccine in Balb/c mice independent of the volume of vaccine utilized to immunize the animals. The results found are very important to reduce the number of doses and the volume of Vero cell rabies vaccine utilized in the immunization against rabies. Financial Support: Butantan Foundation

CO.46
SAFETY AND IMMUNOGENICITY OF THE PURIFIED VERO RABIES VACCINE NEXT GENERATION IN CHINESE PEDIATRIC (≥ 10 YEARS) AND ADULT POPULATIONS

Pichon S[1], Li R[2], Li J[3], Shu JD[1], Chavand P[1], Minutello M[1], Guinet-Morlot F[1] – [1]Sanofi Pasteur, [2]Guangxi Center for Disease Prevention and Control, [3]National Institutes for Food and Drug Control

Background: The Purified Vero cell Rabies Vaccine Next Generation (PVRV-NG) is a highly purified vaccine developed with innovative technology and human and animal origin components-free medium. It was shown to be at least as immunogenic as Verorab™ and presented a similar safety profile in a phase II clinical study conducted in France (pre exposure regimen). A phase III clinical study was performed in Chinese pediatric (≥ 10 years) and adult populations in simulated post-exposure regimen to further document PVRV-NG in comparison to Verorab™. **Methods:** This was a randomized, blind-observer, controlled study in healthy subjects aged 10 to 17 years (pediatric cohort) or ≥ 18 years (adult cohort). Participants received five doses by intramuscular route of PVRV-NG or Verorab™ (ratio 2:1 in each age group) at D0, D3, D7, D14 and D28 as per recommendation for post-exposure prophylaxis (Essen schedule). No rabies immune-globulins were administered concomitantly with the first vaccine dose. Immunogenicity was evaluated at D0, D14 and D42 by measuring the level of rabies virus neutralizing antibodies (RVNA) using the rapid fluorescent focus inhibition test. Testings were performed at the National Institute for Food and Drug Control (Beijing). Safety was evaluated with a list of predefined solicited injection site and systemic reactions during the period between D0 and D14 and during the seven days after the 2 last doses; any adverse events until 28 days after the final dose and any SAE until 6 months after the final dose were also recorded. **Results:** 816 participants were enrolled; 408 in each age group corresponding to 272 in PVRV-NG group and 136 in Verorab™ group. The predefined criterion for noninferiority in terms of proportions of participants with RVNA titers ≥0.5 IU/mL at D14 (before the 4th injection) was met in the per-protocol analysis set and confirmed in the full-analysis set population,

in each of the 2 age groups. All subject had titer ≥ 0.5 IU/mL at D42 whatever the age group and the vaccine received. PVRV-NG was safe and well tolerated after each vaccination and its safety profile was similar to Verorab™ in terms of solicited injection site and systemic reactions, as well as unsolicited adverse events. There were no serious adverse events related to vaccination. No safety signal emerged during the course of the study. **Conclusions:** The phase III clinical study results showed that PVRV-NG is at least as immunogenic as Verorab™ and presented a similar safety profile, when administered according to the ESSEN regimen in pediatric (≥ 10 years) and in adult populations. Taken together with the results of the previous Phase II clinical study, this confirms that PVRV-NG is an improved and highly purified alternative for rabies pre- and postexposure prophylaxis.

CO.47
DEVELOPMENT OF AN IN VITRO ELISA ASSAY FOR THE QUANTIFICATION OF THE IMMUNOGENIC GLYCOPROTEIN G PRESENT IN VACCINE BATCHES: COMPARAISON OF IN VIVO AND IN VITRO VACCINE POTENCY TESTS

Jallet C[1], Castel G[1], Chteoui M[1], Tordo N[2] – [1]Institut Pasteur – Unit Antiviral Strategies, [2]Institut Pasteur – Virology

Since several years, there is a global tendency towards limiting and even sometimes waiving the use of animal experiments in Research and Production. However for Human and Veterinary rabies vaccine producers and controllers, the *in vivo* NIH assay still remains the standard routine potency test before batch release. Nevertheless, fundamental studies have been accumulated to correlate the structural presentation of the main rabies antigen, the glycoprotein G, and its immunogenicity. The G protein contains two main antigenic sites: site II requires a folding of the G ectodomain to bring in proximity peptides distant in the primary sequence of the protein; site III which is less dispersed along the ectodomain but also requires a folded conformation of the ectodomain. Both sites are recognized by specific monoclonal antibodies (mAbs) that are in general neutralizing viral infection. Among them mAb D1 (IgG I isotype), directed against site III is specific of the trimeric state of the glycoprotein (it recognizes the native but not mercapto-ethanol and/or SDS-treated G) which is presumed to be the most immunogenic form of the antigen. mAb D1 has been extensively used to evaluate the stability of G trimers (Jallet et al., 1999, J. Virol, 73: 225-33; Desmezieres et al., 2003, Virus Res. 91: 181-7; Sissoeff et al, 2005, J. Gen. Virol, 86: 2543-52). It has also been proven suitable in ELISA to monitor the consistency of the lot to lot rabies vaccine production and to evaluate the glycoprotein content (Fournier-Caruana et al, 2003, Biologicals, 31 : 9-16). Since the end of the 90t's, the French National Regulatory Authority in charge of human rabies vaccine control (ANSM) has decided to use this ELISA test instead of the single radial immuno-diffusion assay (SRD) to monitor the consistency of production of rabies vaccines. The ELISA has been transferred from Pasteur Institute to ANSM, improved, optimised and then validated. Since 2001, the consistency of production has been established on around 1000 batches while comparing the NIH assay and the ELISA test. The results are homogenous between both methods. In the perspective of replacing *in vivo* by *in vitro* assays, vaccine samples have been artificially altered by heating and the evaluation of the glycoprotein content was assayed by mAb-D1 ELISA. This assay was shown to be sensitive enough to detect vaccine alterations and to discriminate between low and high-potent potency batches. This type of ELISA assay may have a promising future for waiving *in vivo* rabies potency test and promote *in vitro* antigenic/immunogenic quantification/qualification of the G protein for vaccine batches.

CO.48
MOVING TOWARDS THE REPLACEMENT OF THE NIH TEST

Morlot FG[1], CHABAUD-RIOU M[1], CHAPSAL JM[2] – [1]SANOFI PASTEUR – R&D, [2]SANOFI PASTEUR – IO

Potency testing of inactivated rabies vaccines is traditionally performed by an intracerebral (IC) challenge method on mice. The method was originally developed by the National Institutes of Health (NIH) in the 1950s for potency testing of inactivated rabies vaccines for human use. The NIH test is widely recognized and is currently required by the World Health Organization (WHO) and Pharmacopoeias for rabies vaccines release. Nevertheless, the NIH challenge method presents a number of limitation and issues:

- As a biological test the NIH method is highly variable, making this test inappropriate for batch-to-batch consistency analysis.
- A large number of mice is required.
- This test is time consuming and labor intensive for both the manufacturer and the authorities that release the batches. For all these reasons, regulators, experts and manufacturers are seeking an alternative method to the existing NIH test that guarantees the potency of the rabies vaccine to be administered for pre or post exposure vaccination. A recent international workshop of the NICEATM and ICCVAM was held in Ames, Iowa, USA on October 11-13th, 2011 on "Alternative Methods for Human and Veterinary Rabies Vaccine Testing" with particular focus on Rabies Vaccine Potency Testing. The main conclusions of this workshop were as follows:
- For inactivated veterinary rabies vaccines, the Serum Neutralization Test (SNT) serological method described in the Ph. Eur. Monograph 0451, should be immediately considered for product specific validation by vaccine manufacturers for both adjuvanted and non adjuvanted vaccines.
- As human rabies vaccines in some regions (e.g., U.S. and EU) are simpler products (non-adjuvanted, monovalent), manufacturers are encouraged to develop and implement an *in vitro* antigen quantification method to replace the mouse challenge test. *In vitro* antigen quantification methods currently used by rabies vaccine manufacturers as in-process tests include ELISA and Single Radial Immunodiffusion (SRID) Test.
- Final product *in vitro* methods will require identification and use of appropriate reagents (e.g. monoclonal antibody) with specificity for the neutralizing epitope of the virus-associated trimeric form of glycoprotein G.
- Validation of *in vitro* replacement tests will need to include identification of sub-potent lots. For validating *in vitro* methods for potency testing of human rabies vaccines, it may be necessary to compare *in vitro* results to adequate serological titers in humans. In the context of the Purified Vero Rabies Vaccine next generation (PVRV-NG) development, Sanofi Pasteur has set up an in-house ELISA test answering the need for an alternative method to the NIH potency test. The description of the corresponding ELISA method for rabies glycoprotein G quantification and the data supporting the alternative test, together with the proposed global strategy for implementing this ELISA test in replacement of the NIH test, will be presented.

CO.49
AN ELECTROCHEMILUMINESCENT ASSAY FOR ANALYSIS OF RABIES VIRUS GLYCOPROTEIN IN RABIES VACCINES

Smith TG[1], Ellison JA, Carson WC, Ma X, Rupprecht C – [1]Centers for Disease Control and Prevention

Vaccine potency testing is necessary to evaluate the immunogenicity of inactivated rabies virus (RABV) vaccine preparations before human or veterinary