

application. Currently, the NIH test is recommended by the WHO expert committee to evaluate intra- and inter-lot variation of RABV vaccines; however, numerous disadvantages are inherent concerning cost, number of animals and biosafety requirements. As such, numerous *in vitro* methods (e.g. antigen-capture ELISA) have been proposed for the evaluation of vaccines based on RABV glycoprotein (G) quality and quantity which correlates with vaccine potency. In this study an antigen-capture electrochemiluminescent (ECL) assay was developed utilizing three murine anti-RABV G monoclonal antibodies (mAb) to quantify RABV G in two commercially available inactivated RABV vaccines, one experimental vaccine, and three purified RABV G preparations. The first mAb was specific for a conformational epitope so that only immunogenic, natively folded G was captured in the assay. Additionally, two mAbs that bind non-competing linear epitopes were employed to evaluate the overall quantity of native and denatured RABV G and for detection. Vaccine efficacy was also assessed *in vivo* using pre-exposure vaccination of mice followed by peripheral RABV infection. Purified G induced a virus neutralizing antibody (VNA) titer of 4.2 IU/ml and protected 100% of immunized mice; while, an experimental vaccine with low quality and quantity of G induced a VNA titer  $>0.03$  IU/ml and only protected 21% of immunized mice. These preliminary results support the hypothesis that *in vivo* efficacy may be predicted from the *in vitro* measurement of RABV G using the ECL assay. Based upon these results, the ECL assay may have utility in measuring potency of RABV vaccines.

#### CO.50

##### ONRAB® EFFICACY IN SKUNKS (*Mephitis mephitis*) AND RACCOONS (*Procyon lotor*)

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ONRAB®, a recombinant human adenovirus type 5 vector expressing rabies glycoprotein, has been used under experimental permit in the Canadian provinces of Ontario, New Brunswick and Quebec for wildlife rabies oral vaccination programs. Prior to its use in the field, a series of trials were conducted in two terrestrial wildlife vectors to determine the rabies virus neutralizing antibody response to ONRAB®. Eighty-three % of skunks (10/12) and 75% of raccoons (8/12) seroconverted within 6 weeks after consumption of ONRAB® in an Ultralite bait (ULB) at a dose of 109.2 TCID<sub>50</sub>/ml in 1.8 mL. In the subsequent efficacy trial, all skunks (n=28) that consumed a single ONRAB®-ULB were protected from lethal rabies challenge, while 86% (12/14) of the unvaccinated controls succumbed to rabies. In addition, pre-existing neutralizing antibody to either canine adenovirus type 2 or human adenovirus type 5, achieved by intramuscular inoculation of skunks with the viruses 28 d prior to administration of ONRAB® *per os* at 108.4 TCID<sub>50</sub>/ml, had no effect on the antibody response to ONRAB®. These series of experiments demonstrated that ONRAB®-ULB shows promise over previous vaccine/bait combinations as it elicited a measurable immunological response in both skunks and raccoons, and provided protection against experimental lethal rabies virus exposure in skunks. Further, results of these studies suggest that its field performance is unlikely to be affected by pre-existing immunity to other adenoviruses.

#### CO.51

##### PRODUCTION AND EVALUATION OF A CHROMATOGRAPHICALLY PURIFIED VERO CELL RABIES VACCINE (PVRV) IN CHINA USING MICROCARRIER TECHNOLOGY

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China is a high population country with millions of animal bite cases every year; thus, it is necessary to explore and develop more effective and productive rabies vaccines for human use. To establish a safe, effective, inexpensive and high-yield rabies vaccine, a non-adjuvant purified Vero cell rabies vaccine produced in the SPEEDA PVRV microcarrier bioreactor was developed by Liaoning Chengda Biology Co. Ltd. in China. This vaccine was produced using Vero cells that were cultured in a microcarrier bioreactor. A microcarrier bioreactor containing 25 g/L of Cytodex-1 was used for perfusion culture. The Vero cell culture density was up to 1.2–1.5 × 10<sup>7</sup> cells/ml, viruses could be constantly harvested for 18–22 d, and the resulting vaccine immunizing potency was  $\geq 4.5$  IU/ml. Vaccine safety and immunogenicity post-immunization were also assessed. A total of 602 volunteers were enrolled and divided into two groups that were vaccinated with either SPEEDA PVRV or VERORAB PVRV on days 0, 3, 7, 14 and 28. All subjects vaccinated with SPEEDA PVRV showed no serious local or systemic adverse effects. The positive conversion rate of serum neutralizing antibodies against the rabies virus reached 100% in both the test and control groups (inoculated with VERORAB PVRV) at 14 d and 45 d after vaccination, and no significant difference was found between the neutralizing antibody geometric mean titers (GMTs) of the two groups. SPEEDA PVRV is appropriate for mass production and shows satisfactory clinical safety and immunogenicity for human post-exposure prophylaxis of rabies.

#### CO.52

##### SKUNK RABIES IN TEXAS; A RETROSPECTIVE LOOK

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Skunk rabies in Texas, USA is ubiquitous, with the majority of the state within the range of the South-Central skunk rabies distribution. Statewide public health surveillance indicates a cyclic trend, with peaks in total skunk rabies cases approximately 22 years apart. We examined public health case-reports from 1960-2006 to identify trends, with the ultimate goal of developing a predictive model for skunk rabies epizootics. Cases were plotted by county, by year and certain trends were observed. Some counties regularly reported skunk rabies cases while many others reported no cases for several years. We also examined rainfall data from 4 representative counties to determine if there was a correlation between rainfall and skunk rabies cases. This paper presents the results of these investigations and presents opportunities for further investigations.