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CO.01
REAL TIME PCR FOR ANTEMORTEM DIAGNOSIS IN HUMANS AND HIGH THROUGHPUT RABIES VIRUS SCREENING

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Rabies is one of the oldest and most devastating diseases, typically a 100% case mortality rate. *Antemortem* rabies diagnosis in humans is important to institute infection control procedures and avoid further exposures of health personnel and other people who may have been in contact with saliva of the patient, to determine epidemiologically if other individuals were exposed to the same source, to monitor disease progression during experimental therapeutic interventions, to prevent cross infection during organ transplantation, and if negative, to examine other differential diagnoses. Four samples are recommended for *antemortem* testing of rabies in humans (saliva, serum, nuchal skin biopsy and cerebrospinal fluid) in which antibodies, viral antigens and nucleic acids are detected using neutralization, indirect and direct immunofluorescence and reverse transcription PCR methods. The turnaround time to provide conclusive results on all four samples analyzed by all techniques is approximately 24 to 48 hrs. Real time PCR appears to be one promising technique to expedite results on nucleic acid detection and accurately quantitate viral loads in patient samples. However, there is no universal primer or probe that may detect the broad lyssavirus diversity described to date, which decreases the sensitivity of the test dramatically. The objective of this study was the design of a sensitive and specific real-time PCR assay to detect and quantitate a broad variety of rabies viruses in *antemortem* and postmortem samples from human/animal tissues and bodily fluids. **Methods**. More than 2500 complete nucleoprotein sequences of RABV representative of a global spectrum of variants from the GenBank and CDC databases were analyzed to design 12 sets of broadly reactive primers and probes. Two primer sets and probes from the literature were concomitantly considered. A total of 14 primer sets and probes were tested both in silicon and with a panel of 20 viral isolates (representing a broad variety of RABV circulating in bat and carnivores worldwide) using end point reverse transcription PCR and real time PCR assays run in both a Light Cycler 480 (Roche, Germany) and CFX96 Touch™ Real-Time PCR (Bio-Rad, USA) Detection System for comparative purposes. A droplet digital PCR system QX100 (Bio-Rad Laboratories, Inc) was used to assess the number of copies of amplicons per sample. To confirm detection, PCR products were sequenced and identified in the BLAST NCBI database. **Results.** The prior primer sets and probes from the literature had limited sensitivity circumscribed to RABV circulating in respective regions of Asia and Africa. None of the primer sets and probes we designed was able to detect all viruses tested alone. However, a minimal set of five primers and probes used concomitantly in separate reactions were able to detect all RABV included in this study. The system was sensitive enough to detect up to 5 copies of amplicon. **Conclusions**. Real time PCR is a highly sensitive and specific technique yet there is no single set of broadly reactive primers and probes capable of detecting all the existing lyssavirus diversity. We described five broadly reactive primer sets that were used concomitantly in a 96 well platform to detect all RABV reported globally. This format is suitable for rabies *antemortem* diagnosis in humans and for high throughput screening of field samples. Additional research on a quantitative assay will not only be able to assess the number of copies of amplicons, but also correlate with an estimated total number of viable viral particles. **Reference** Wacharapluesadee S, *et al*. Expert Rev Mol Diagn. 10(2):207, 2010. Coertse J, *et al*. J Clin Microbiol. 48(11):3949, 2010.

CO.03
MONITORING OF A FRENCH BAT COLONY SHOWN NATURALLY INFECTED BY EBLV-1 FROM 2009-2012. DISCOVERY OF A NEW INFECTION CASE IN THE COLONY THREE YEARS AFTER THE FIRST POSITIVE RABIES DIAGNOSTIC.

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The passive surveillance of bat rabies was improved in France since 2000, thanks to a National bat rabies network constituted by Veterinary Services and bat handlers from the Chiroptera group (Société Française pour l'Etude et la Protection des Mammifères). To date,59 *Eptesicus serotinus* were shown infected by the European Bat Lyssavirus type 1 (EBLV-1) in France out of 950 cases reported throughout Europe. In the context of the passive surveillance, we reported for the first time in June 2009 in France, in the village of Ancy sur Moselle (located in the north east of the country), six positive cases in a colony of *Eptesicus serotinus* naturally shown infected by EBLV-1. The field studies, carried out from 2009-2012 on 186 tested bats with vaccinated bat specialists showed a fall of the seroprevalence by 5. In 2009, 45% blood samples were shown positive with a modified FAVNt adapted for bat micro-samples for the presence of neutralizing EBLV-1 antibodies against 8% in 2011. Three years after the first report of the EBLV-1 infection in the colony of Ancy Sur Moselle, a new positive case was reported into the reproduction colony at the end of July 2012. The colony was constituted by 80 bats in May and by 46 animals at the end of July. Similarly to 2009, the case was reported on a juvenile female and the isolated virus was EBLV-1b. Thanks to the education of bat workers to the risks of bat rabies, a new positive case was detected on July 23, 2012 in Eastern France on a *Myotis naterreri.* This species was recently shown infected by BBLV (Bokeloh Bat Lyssavirus) in Germany. To date, this is the first case reported in France on the species *Myotis naterreri.* This case was found in a village distant of 40 km from Ancy sur Moselle. The monitoring of the colony from Ancy sur Moselle with the results of field studies investigated from 2009-2012 will be presented and discussed as well as the new case of infection diagnosed on *Myotis naterreri.*