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Session: New Insights on Rickettsial Infections
Date: Thursday, March 3, 2016
Time: 15:45-17:45
Room: G.01-03

Rickettsia felis burden in the tropics

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Abstract: *Rickettsia felis* bacterium has been observed since the early twentieth century in the cat flea. It was grown for the first time in my laboratory on cell Xenopus then insect cells particularly *Aedes albopictus* cells. It grows only at temperature under 30 °C. First described in California, the bacterium is present in the whole world. For a long time it was considered a rare disease associated with fleas. In fact, in recent years, *Rickettsia felis* was found as the most frequently identified bacteria in patients with fever in tropical countries after malaria. These data were confirmed in West Africa and East Africa by different teams. *Rickettsia felis* has also been detected in tropical and tropical areas of South America, Asia and Oceania. *Rickettsia felis* and is the most common rickettsial currently in the world and very frequently associated with fever in the tropics and cough but no rash. In contrast controls without fever, can cause at a lower but significant level, the presence of *Rickettsia felis* DNA. So these circulations of *Rickettsia felis* in the blood that are not always associated with fever. Possible vectors in tropical areas may be mosquitoes in Africa. *Rickettsia felis* has the same epidemiological distribution as malaria. *Rickettsia felis* was found in *Anopheles* species and *Aedes albopictus. A* mouse experimental model was established showing that *Rickettsia felis* could be vectorized by Anopheles gambiae. *Rickettsia felis*, finally, recently, was found in book lice in the dust, which further complicates the epidemiological cycle of this bacterium. *R. felis may be* the most common bacteria emerging

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Expression of glycoprotein gene of Rabies virus and evaluation of recombinant protein for seromonitoring of vaccinal antibodies in dogs

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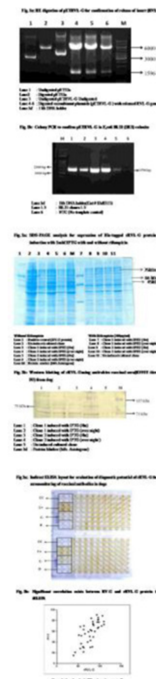
Background: In India, control of rabies in dogs by massvaccination is the practical approach. Currently, viable infectious Rabies virus (RABV) dependent and laborious Rapid Fluorescent Focus Inhibition Test (RFFIT) is employed in selected laboratories in India to monitor the vaccinal antibodies. This situation demands an alternative rapid, sensitive, specific and user friendly test. Hence, this study was undertaken to express the recombinant glycoprotein (rRVL-G) of Dr. Larghi's strain of RABV and evaluate it's diagnostic potential.

Methods & Materials: The RABV propagated in BHK 21 cells was used as the source of G gene. The Polymerase Chain Reaction (PCR) product of G-gene was cloned into pGEM®-T Easy Vector and transferred into competent Top 10 *E. coli*. The insert from the recombinants (pGRVL-G) was subcloned into pET32a vector, transferred into TOP 10 cells and recombinants (pETRVL-G) obtained. These recombinants were transferred into *E.coli* BL21 and screened for expression and immunogenicity by SDS-PAGE and Western Blotting respectively. Indirect ELISA was standardized by Checkerboard titration of complete RABV-G protein (RV-G) and diagnostic potentials of rRVL-G confirmed.

Results: The PCR product of RABV complete G gene revealed a band of 1596 bp. The PCR product cloned in pGEM®-T Easy Vector and subbcloned in pET32a vector was confirmed by Restriction Enzyme (RE) digestion and colony PCR (Fig. 1a, 1b) and sequencing. Further SDS-PAGE of the IPTG induced clones revealed a fusionprotein of 75 kDa which was confirmed to be immunogenic by Western blotting (Fig. 2a, 2b) when probed with anti rabies vaccinal dog sera. Standardization of indirect ELISA and application using RV-G and rRVL-G by testing of 40 anti rabies vaccinal dog sera of varying RFFIT titres revealed a significant correlation in the performance of both the antigens (Fig. 3a, 3b).



Conclusion: The outcome of the work suggested the diagnostic potentials of the recombinant protein in ELISA for seromonitoring of antirabies vaccinal antibodies. In view of the availability of ELISA facilities, expertise and limitations of RFFIT in India, the results encourage the development of recombinant G-protein based ELISA as a newer diagnostics for sero monitoring of antirabies vaccinal antibodies in both domestic and street dogs at a regular interval of time.

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