Executive Summary of UGC project for pacing on Institutional Website

1. UGC Reference No : **F No. 42-634/2013 (SR)**

2. Period of report: **from 01.04.2013 to 31.03.2017**

3. Title of research project: **Development of control strategies based on molecular epidemiology and drug efficacy for equine piroplasmosis in Punjab**.

4. (a) Name of the Principal Investigator: **Dr. Lachhman DasSingla**

   (b) Deptt.: **Veterinary Parasitology**

   (c) University/College where work has progressed: **College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana**

5. Effective date of starting of the project: **01.04.2013**

6. Grant approved and expenditure incurred during the period of the report:
   a. Total amount approved Rs. **Amount allocated = 8,20,000/-; Amount Sanctioned = 7,45,000/-**
   b. Total expenditure Rs. **7,42, 669/-**
   c. Report of the work done: (**Separate sheet attached as Annexure –I)**

7. Brief objective of the project

   1. Development and standardization of accurate, sensitive and specific PCR based diagnostic for *B. equi* and *B. caballi* in equines.

   2. Evaluation of molecular epidemiology and drug sensitivity based on PCR techniques in equine piroplasmosis to develop control strategies.

8. Human resource

- **a) Manpower trained**: one Ph.D. , One M.V.Sc and Research training Fellow
- **b) Ph.D. awarded**: one
- **c) Publications of results**: 9 papers published + 3 under preparation + 3 papers presented at national and international conferences and abstracts published + 5 genomic sequences submitted in NCBI database + 2 review articles + 2 book chapters
- **d) Other impact**: The methodologies/protocols standardized in present study can be used by students, researchers and others scientist for know the molecular epidemiology and drug testing studies.

9. SUMMARY OF THE FINDINGS

The equines (horses and donkeys/mules) from from five agro-climatic zones of Punjab were screened to explore the prevalence of Theileriosis caused by *Theileria equi* an important haemoprotozoan parasite of phylum Apicomplexa, order Piroplasmida. Various diagnostic tests were optimized and employed on the blood samples for the assurance of results including molecular tools by selecting more specific primers and better DNA extraction method for high sensitivity and specificity. The three important diagnostic procedure viz., microscopic, molecular (18S rRNA) and serological, revealed an overall prevalence of 3.66% by blood film, 11.64% by primary PCR, 21.77% by nested PCR and 49.78% by indirect-ELISA (EMA_2), with an increasing trend from northeast to south west. Overall highest prevalence was recorded in Western Plain Zone by all four techniques with the detection rate of 9.52, 22.22, 42.86 and 80.95% respectively. District wise highest prevalence was seen in district Fazilka by microscopic (11.53%), primary PCR (23.07%), indirect-ELISA (84.62%), and in Ferozepur by nested PCR

(43.24%) tests. The assessment of various physical and biological risk factors included the presence of tick vector (OR=5.13) followed by farm management system (OR=3.23) species type (OR=2.87), sex of the host (OR=1.80), as the most influential factor for infection of *T. equi* infection. Evaluation of haemato-biochemical parameters showed significant increase in TLC, MCH, GLO, CRCS, AST, GGT, TBIL and decreases TEC, Hb, PCV parameters in equines positive by blood film and primary PCR as compared to non infected group. While nested PCR and indirect-ELISA revealed significant increase in CRSC, GGT, TBIL, DBIL, GLU and decrease in TEC as compared to non infected group. SEM revealed characteristic identification features of ticks collected, which upon analysis by PCR assay revealed the presence of *T. equi* parasite in 11 out 84 specimens. Incidence of parasite was higher in *Hyalomma anatolicum anatolicum* (14.71%) as compared to *Rhipicephalus (Boophilus) microplus* (6.25%), particularly the female ticks (Odds ratio=4.71). Multiplex PCRs were optimized and employed on representative samples as a time and cost effective tool for co-infection of *T. equi* and *T. evansi* as well as *T. equi* and *B. caballi.* As no case for *B. caballi* was recorded by multiplex PCR, these results were further corroborated with cELISA (EMA_1) which revealed 75% and only 1.11% prevalence of *T. equi* and *B. caballi*. The nucleotide sequences obtained from the PCR amplicons of *T. equi* in the present study showed 99% homology to the Brazil isolates. Western Plain Zone is more prone to *T. equi* so control programme should start from these areas.

The drug efficacy testing was done by testing the *T. equi* positive blood samples before and after chemotherapy with the help of microscopic method &PCR assay. The haematological values were also compared before and after chemotherapy in positive animals. Microscopically all treated animals were found negative for *T. equi* infection. However; by PCR assay it was found that buparvaquone was 100% efficacious in eliminating the parasitic DNA from the host blood whereas diminazene aceturate was comparatively less efficacious.

Present study is the first report on molecular and serological epidemiology of *T. equi* establishing the endemic stability of this infection in Punjab. The subclinical infections without the predominant signs of theileriosis can also affect the health of equines as revealed from the apparent changes in clinico-hematobiochemical parameters. Western Plain Zone is more prone to *T. equi* infection so control programs should start from these areas and should be carried out in upward direction covering whole Punjab.

10. CONTRIBUTION TO THE SOCIETY

The clinical incidence of equine piroplasmosis is more common in foreign breeds of horses kept in enzootic zones however the latent infection is common in non-descript equines especially in India. The clinical form of the disease is diagnosed by peripheral blood smear examination, but in carrier animals it is very difficult to demonstrate the parasite in stained blood smears as the parasitaemia is extremely low. For diagnosis of such low grade infection or carrier animals, serological tests and DNA-based molecular diagnostic techniques have become mandatory now so that the dreadful diseases can diagnosed early and the preventive measure can be taken thereof . The developed and standardized molecular methods will prove to be helpful in diagnosing the sensitivity of available drugs based on the negative or positive DNA signals obtained after treatment. Thus may also be helpful in prevention of transfer of the parasite to foals from pregnant mares for adopting control strategy. Thus PCR protocls may prove to be useful in the diagnosis of equine babesiosis covering a wider

range of clinical disease, as useful adjuncts to serological, microscopic, and cultural methods, especially for the import and export testing of horses and to test the drug sensitivity in order to develop control strategy.

This project has helped in determining the current endemic epidemiological situation concerning the equine piroplasmosis. The standardized protocols will be of great help for promptly diagnosing latent infections which may act as nidus for spreading the diseases to other susceptible equines. Further will help in controlling equine piroplasmosis in a better manner by applying better diagnostic tools and thus will help in providing better health coverage to equines of Punjab state. The clinicians and scientists throughout the country can use the standardized protocols developed for the clinical, parasitological and molecular diagnosis of equine piroplasmosis.