Summary
VI. SUMMARY

The present study was undertaken with the objectives of isolation of Bovine herpes virus -1 (BoHV-1) from semen and clinically suspected samples in cell culture system, screening of serum samples by Indirect ELISA, molecular characterisation and phylogenetic analysis by cloning and sequencing of partial glycoprotein B gene of BoHV-1 isolates. The study was aimed at standardisation of Real time PCR targeting glycoprotein B gene of BoHV-1, comparative evaluation of Real time PCR with Virus isolation and Indirect ELISA and development and validation of Real time PCR based antigen detection kit for diagnosis of BoHV-1. The summary of the present study is as follows:

Samples were collected from 212 animals from four organized farms and BoHV-1 suspected outbreaks under field conditions.

Seroprevalence of BoHV-1 as studied Avidin - Biotin ELISA kits supplied by PD_ADMAS, Bangalore. Indicated 34.90 % of the animals tested during the study as seropositive for BoHV-1 antibodies. Farm B which maintained breeding animals in large, open animal sheds, in let loose conditions with common grazing and drinking areas recorded the highest seroprevalence of 56 % during this study.

The least seroprevalence of 17.75 % was recorded in Farm D which had better managemental, hygienic and bio-security practices with spacious modern individual animal pens, separate feeding and drinking facilities within the pen.
Swab samples collected from 121 cows and neat semen from 91 breeding bulls were subjected for virus isolation in MDBK cells. Swab samples from eight female cattle and semen from one bull yielded characteristic cytopathic changes suggestive of BoHV-1 in cell culture.

The virus isolates were confirmed by virus neutralization test. All the BoHV-1 isolates were completely neutralized by BoHV-1 hyper immune serum obtained from PD_ADMAS with neutralization index of more than 1.5.

Samples from five out of thirty five animals showing classical symptoms of the disease under field outbreaks yielded the virus in cell culture system.

A conventional PCR targeting a conserved region of 443 bp on the gB gene of BoHV-1 was selected for molecular confirmation of the isolates. The gB specific PCR amplicons from nine isolates obtained during this study were inserted in pGEM-T easy vector, cloned in JM 109 high efficiency cells. The clones were checked for desired insert by Eco R1 restriction enzyme digestion. The nucleotides were sequenced, aligned and Phylogenetic analyses were conducted using MEGA version 5 using the maximum parsimony method with 500 bootstrap replicates.

Based on the nucleotide sequences, five of these nine isolates KVAFSU BNG-1, KVAFSU BNG-2, KVAFSU BNG-3, KVAFSU BNG-4 and KVAFSU BNG-9 were clustered under BoHV-1.1 subtype. These isolates were more than 99.1 % identical with reference PD_ADMAS strain and Switzerland strain of BoHV-1.
The other four isolates KVAFSU BNG-5, KVAFSU BNG-6, KVAFSU BNG-7 and KVAFSU BNG-8 were grouped under BoHV-1.2 subtype. These were 100% homologous between themselves and shared a sequence identity of 100% with an European strain of BoHV 1.2 and 98.9% sequence identity with PD_ADMAS BoHV-1.1 isolate.

A TaqMan Real time PCR assay was standardized for the detection of BoHV-1 including subtype 1 and 2 in clinical samples including semen. A set of primers and TaqMan probe targeting selective amplification of a 71 bp sequence of the conserved glycoprotein B gene of BoHV-1 were designed. Optimization of different components of real time PCR during this study was done through checkerboard titration method.

The Real time PCR assay successfully detected both BoHV-1 subtypes 1.1 and 1.2 isolated during this study. The detection limit was determined as 0.001 TCID$_{50}$ of the virus in tissue culture supernatants. The real time assay was able to detect dilutions of positive semen sample of up to a dilution of 1:80, while virus isolation performed on these dilutions of semen samples yielded the virus only in 1:10 dilution.

The developed real time PCR assay was 100% sensitive and 87.17% specific in detection of BoHV-1 in clinical samples in relation to virus isolation.

Real time PCR when applied on 212 clinical samples collected in this study, the assay detected virus in all the samples that yielded virus in cell culture indicator system, apart from detecting twenty six additional samples as positive for BoHV-1. Hence the
study finds that the real time PCR is more sensitive test for virus detection than virus isolation method.

Out of 74 seropositive animals, four animals in virus isolation and 22 animals in real time PCR indicated presence of BoHV-1. Five out of 138 seronegative animals yielded virus in MDBK cell line during virus isolation and qRT PCR detected BoHV-1 in thirteen seronegative animals.

A TaqMan Real time PCR based antigen detection kit was developed and the kit was validated at NCBS, Bangalore; Bigtec labs, Bangalore and at PD_ADMAS, Bangalore with ≥ 99 per cent sensitivity of the developed kit.