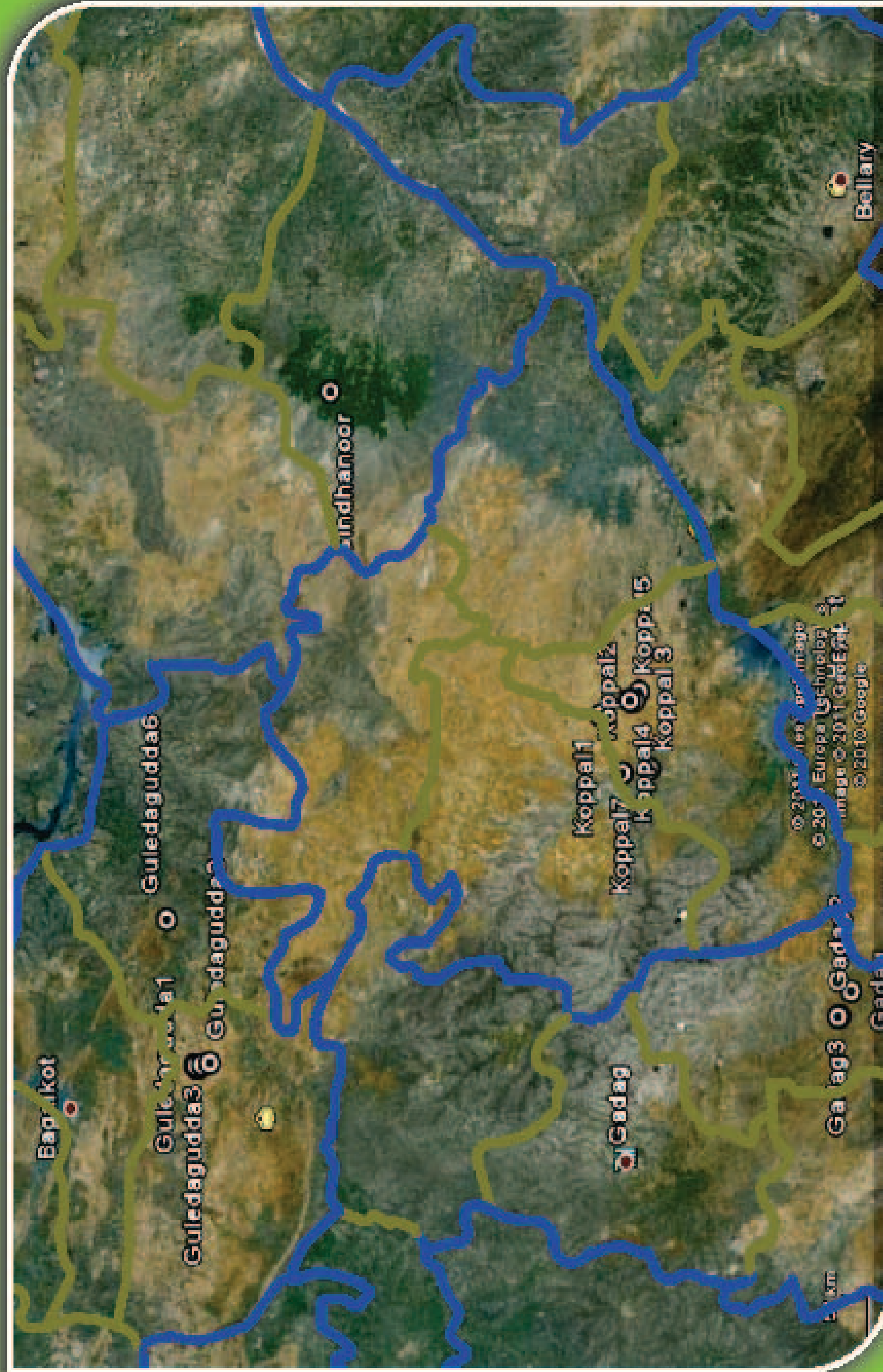
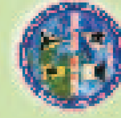


Project Directorate on Animal Disease Monitoring and Surveillance



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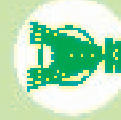

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Back Cover :
Satellite image showing the locations of
Bluetongue outbreaks in five districts of Karnataka (2009)

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Jai Hind

(H. Rahman)
Project Director

Executive Summary

Establishment of early warning surveillance systems, preparing for, investigating and responding to priority diseases is very much critical in reducing morbidity and mortality in vulnerable populations, keeping in view of protection of global health security. Delay in the detection of outbreaks and inadequate preparedness and response aggravates the impact of spread of diseases, leading to increased number of cases, increased duration of epidemics, excess mortality and the potential for spread to other areas nationally, regionally, or globally. Project Directorate on Animal Disease Monitoring and Surveillance (PD_ADMAS) is only institute catering to the needs of surveillance and motioring of livestock diseases and thereby caring for the country's animal health. Livestock population and disease profiles available in the databank are the cynosure of the institute. Different units of PD_ADMAS are working towards designing of various forecasting and forewarning modules in order to predict the livestock disease outbreaks. Spatial epidemiology, temporal epidemiology, local and global epidemiology, molecular epidemiology of various livestock diseases are routinely studied which are of utmost important in formulating the disease control strategies. The institute has also developed spreadsheet modules for economic impact analyses of various diseases.

The backbone of the Directorate is epidemiology unit which has two sets of data, Static and Dynamic. The disease profile data consists of number of attacks, susceptible animals, deaths and vacinations with respect to each disease in a particular district with species of the animals involved, year and month of the outbreak. At present the data bank has a well documented 77,777 records pertaining to various livestock disease outbreak reports of the country from 1987 to 2011. The dynamic data in the databank is updated regularly after suitable validation. On analysis of the data it was noticed that FMD, HS, and Babesiosis are the top viral, bacterial and parasitic diseases respectively reported from the country. The ecopathozones for economically important diseases viz., BQ and HS were prepared. A logistic regression analysis for 15 economically important livestock diseases has been carried out using NADRES model. Based on the predicted group values obtained in the logistic regression model, forecast maps for different disease were prepared for the 12 calendar months. By spatial epidemiology, it is evident that haemorrhagic septicaemia tops the outbreak reports followed by black quarter and have been reported from almost all the states, throughout the year. Spatial analysis of FMD, PPR, theileria, trypanasoma and babesia and one gastrointestinal (Fasciola) parasites have been studied. In temporal epidemiology, some of the major livestock diseases were studied at zonal level and the study is based on ten years' (2001 - 2010) data available in the databank. It was noticed that all the six zones (North, South, East, West, Central and North-East) report HS over the period whereas for BQ and Anthrax there is relatively less reports. The results also indicate that in the South zone the disease has been consistently recorded whereas in North-East zone there is low or negligible reporting of the disease.

Analysis also showed that the disease has been more recorded in cattle than in buffaloes. Similar studies on PPR showed that initially all the zones reported the disease upto the year 2003 and later on the reports dwindled to the extent that by 2010 only South Zone and West Zone reported the disease. The South Zone showed a consistent disease incidence and the other zones showed nil to negligible incidences. Spreadsheet modules for economic impact analyses for different diseases are prepared to estimate the 'direct costs' (output loss/resource wastage, treatment and prevention costs) of each diesase. Economics on reproductive disorders in bovines of organized farms vis-a-vis to nutritional status showed decreased levels of zinc traces in animals with reproductive problems.

First of its kind in repository systems available in the country is the National Livestock Serum Repository which caters to needs of the population epidemiology. The serum samples of five livestock species (cattle, buffaloes, sheep, goats and pigs) were collected by stratified random sampling method and deposited at the repository by the collaborating units of the Directorate. During the year 2010-11 a total of 2466 serum samples from eight livestock species were received. As part from this study, 1085 cattle sera samples referred to the Directorate for either screening or confirmation for brucellosis were subjected to

AB-ELISA and 67% of them were found to be positive for the antibodies.

Brucellosis a reproductive disease of animals is also of zoonotic importance causing chronic debilitating disease and infertility in man. Various diagnositc tests/ assays were developed for diagnosis of brucellosis in livestock and humans. The indirect ELISA developed was applied to screen anti-brucella antibodies in goats (n=2362) and sheep (n=1702) samples of different states of India. The highest seroprevalence was recorded in goats of Madhya Pradesh and Bihar and in sheep population of Karnataka and Rajasthan. The iELISA was evaluated with representative sero-positive and negative sheep and goat blood and sera by isolation and PCR. Provisional patent NO. 01592/CHE/2008 for the iELISA has been obtained. There is a repository of *Brucella* isolates. Species identification of *Brucella* isolates by multiples Bruce ladder and AMOS PCR was made. Phylogenetic tree of *Brucella* isolates-OMP-2b gene based sequences were analysed and studied. Common diagnostic test developed for diagnosis of brucellosis in human and livestock is an important achievement. Recombinant bp26 protein based ELISA for diagnosis of human brucellosis is an added facility of the institute.

Under the outreach programme on zoonotic diseases, epidemiology of brucellosis, leptospirosis and listeriosis in addition to their zoonotic relevance were studied. The burden of these agents in livestock and their products is documented. Interestingly, among pig samples 51.2%, 74.35% and 20.51% were positive for brucellosis by RBPT, ELISA and PCR, respectively indicating the higher prevalence of both antibody and antigen in pig samples. Different livestock species including 11 tiger and equine samples were screened for *Leptospira* spp both by PCR and isolation methods. Four out of 10 cattle and 11 out of 16 goat serum samples were positive. Zoonotic potential of brucellosis, leptospirosis and listeriosis in samples from human were collected from risk group (veterinarians, para veterinarians, farmers/workers associated with the animals) and persons showing clinical signs were assessed.

Molecular epidemiology of food-borne pathogens of livestock and livestock products has revealed many important findings. The BURST analysis revealed that there was one major lineage within this collection of isolates (the ST-7 clonal comples) which contained 34 STs with St-12 as a single locus variant of ST-7, which suggests it may be well fitted to listerial infection. The data generated from the *L. monocytogenes* isolates in the present study provides a frame work upon which the distribution of genes involved in pathogenicity can be superimposed and that can be expanded in future studies to improve understanding of the population structure and global epidemiology of this pathogen.

PD_ADMAS has a unique place in the field of Leptosira research in the country. The research activities in leptospirosis since inception of PD_ADMAS has led to isolation of a large number of leptospira isolates from diverse animal and human hosts and development of a simple leptospiral staining kit. Therefore, it is imperative to identify and characterrize the *Leptospira* isolates from livestock and human from time to time for monitoring of leptospirosis particularly in livestock in the country. This in turn will provide the facility for typing within the country and selection of panel of antigens to be used in the MAT diagnositc method. Zoonotic significance of *Leptospira* spp serovar Hardjo is particularly significant. Infected cattle are known to be maintenance hosts resulting in illness leading to abortion, infertility and mastitis and their calves may be still born, weak or clinically normal but harbour infection as a potential source to in contact animals and human. The standardization of immunoassay using rLigB/IgG like protein of *Leptospira* spp is underway which would differentiate pathogenic and non-pathogenic leptospiral infections in cattle herd.

The institute has a facility to diagnose the carrier status of Trypanosomiasis in bovines and the development of recombinant VSG protein based ELISA for detection of antibodies against *T.evansi* is under standardization. Ecopathozone maps for important parasitic diseases e.g. trypasomiasis, theileriosis, babesiosis, fascioliasis have been created.

Extensive and in depth study on epidemiology of mastitis is undertaken with special reference to local and global epidemiology of pathogens and their factors associated in causation of mastitis (under NAIP lead project). The institute is equipped with modern tools for such studies. Milk samples collected from dairy farms were processed for the isolation and identification of major mastitic pathogens. From 85 milk samples, a total of 205 orgnisms were isolated

based on colony morphology and other cultural characteristics including colour, shape, size, etc. and identified by genus and species specific PCR/mPCR assay standardized earlier. The most predominant pathogens were *Staphylococcus* spp. (71.70%) followed by *Streptococcus* spp. (14.15%) and *E. coli* (14.15%). Molecular epidemiology of *Staphyococcus* revealed that none of the *S.aureus* isolates belonged to ST 398 which is an importnat factor in zoonosis. The correlation between prevalence of mastitis pathogens and its relation to antibiotic/methiciliin resitance studies will be of much helpful in diagnosis of subclinical mastitis. It is an achievement that a quantitative multiplex PCR was standardized for detection of 14 mastitis pathogens.

Of the 2609 serum samples (Goat-92; Sheep-356, Cattle-2161) screened for PPRV antibodies by using PPR C-ELISA kit, the percentage prevalence of 23.31, 9.78 and 4.79 were observed in sheep, goat and cattle, respectively. A total of 660 serum samples (Goat -265; Sheep-317; Bovine/others-78) were screened by collaborating institute, for PPRV antibodies by using PPR C-ELISA kit. The percentage positivity of 43.3, 53.9 and 26.9 were observed in goat, sheep, and bovine, respectively. Of the 268 clinical smples (Goat-190; Sheep-75; bovine/other-2) screened for PPRV antigen by using PPR S-ELISA kit, the percentage positivity of 26.3 and 44 were observed in goat and sheep, respectively. Seroprevalence of PPR in cattle is at the basal level. No significant difference was observed between the cattle and buffaloes in the percentage positivity for PPR antibodies. High significant difference between the sheep and goat samples was observed.

The detailed epidemiology on bluetongue disease was started in 2009 (as part of AINPBT). The information about suspected outbreaks was received by the PD_ADMAS unit from Bidar, Raichur and Gadag districts of Karnataka. A quick look at the trend line indicates a marginal decrease in the number of bluetongue outbreaks in the state compared to the previous year. Interestingly, the number of affected animals is slightly less than half of the number affected the previous year and so was the case with number of deaths resulted from the disease. This has anything to do with strain/serotype/flock immunity needs to be explored. Spatial epidemiology of BT using GPS and GIS coordinates has been helpful in identification of epidemiological factors associated with the disease prevalence on maps. The preliminary analysis indicated that clinical samples collected from Bagalkot district were positive for serotype 1 and those obtained from Sindhanoor were positive for serotype 2 and 1. Analysis of 677 serum samples showed that 185 (27.32%) were positive for anti-BTV antibodies. Study on correlation between BTV and *Culicoides* spp are also underway using indigenously developed insect trap.

In seroepidemiology of IBR, 57009 serum samples from different parts of the country were tested by AB-ELISA for IBR antibodies during 1995-2010 and 20749 (36%) were found positive. The variation in the overall prevalence of IBR may be attributed to the sample size. In molecular epidemiology of BoHV-1 infection, during this year, eight IBR/IPV suspected clinical samples collected from cattle/buffaloes along with three other isolates (ADMAS-1, ADMAS/258-08 and Ooty) in our repository were processed for viral DNA extraction, PCR and partial nucleotide sequencing of the gB gene. Aligned nucleotide sequences revealed high degrees of identity in all alpha herpesviruses compared in the study. The nucleotide sequence identity among the Indian strains varied from 99-99.5%, so was their identiy with reference Cooper strain. All the Indian strains used in the study, belong to subtype 1.1 irrespective of animal species and clinical manifestations. Taken together, the phylogenetic analysis indicates that BoHV1.1 is the subtype prevalent in India.

During this year, seroprevalence of classical swine fever was conducted and a total of 1257 serum samples collected from four (4) states viz., Andhra Pradesh, Karnataka, Kerala and Maharashtra were subjected to blocking ELISA using commercial kit. A total of 237 serum samples were positive for CSF antibodies. Andhra Pradesh (19%) recorded a highest percent prevalence of CSF antibodies, followed by Kerala (22%), Maharashtra (14%) and Karnataka (12%).

To conclude, PD_ADMAS will be playing key role in national livestock disease monitoring and surveillance to secure animal health of the nation.

कार्यकारी सारांश

वैश्विक स्वास्थ्य सिक्युरिटी को ध्यान में रखते हुए, संकट-ग्रस्त स्थानों में अस्वस्थता एवं मृत्यु-दर को कम करने में, प्रारम्भिक चेतावनी निगरानी पद्धतियों की स्थापना, प्राथमिकता वाले पशु-रोगों की छानबीन एवं रोग-नियंत्रात्मक तैयारी बहुत ही आलोचनात्मक है । रोग-प्रस्फोटों की जांच में देरी एवं अपर्याप्त तैयारी एवं प्रतिक्रिया, रोगों के फैलने में मदद करते हैं, जिसके परिणाम-स्वरूप रोग के केसों की संख्या में बढोत्तरी, महामारी की अवधि में बढोत्तरी, मृत्यु-दर में बढोत्तरी हो जाती है एवं क्षेत्रीय-स्तर पर, राष्ट्रीय स्तर पर और वैश्विक स्तर पर अन्य क्षेत्रों में रोग-फैलने की संभावनाएं भी बढ जाती है । पशु-रोग संवीक्षण एवं निगरानी पर कार्यरत परियोजना निदेशालय (पीडी-एडमास) अपने तरह का एक अकेला संस्थान है, जो पशुधन रोगों के संवीक्षण एवं निगरानी सम्बन्धित आवश्यकताओं को पूरा करता है और देश के पशु-स्वास्थ्य की जरूरतों से सम्बन्धित देखभाल करता है । डेटा-बैंक में उपलब्ध पशु-धन जनसंख्या एवं रोग-प्रोफाइल्स, इस परियोजना निदेशालय के मुख्याधार हैं । पशुधन के रोग-प्रस्फोटों की भविष्यवाणियां करने के दृष्टिकोण से, परियोजना निदेशायल (पीडी-एडमास) की अन्य-दूसरी इकाईयां, विभिन्न भविष्यवाणियों एवं पूर्व-चेतावनी मोडयूल्स के डिजाइन तैयार करने में कार्यरत हैं । विभिन्न पशुधन रोगों का आकाशीय महामारी विज्ञान, समायिक महामारी विज्ञान, स्थानीय एवं वैश्विक महामारी विज्ञान, आण्विक महामारी विज्ञान आदि का प्राय: आवश्यकतानुसार अध्ययन किया जाता है, जोकि रोग-नियंत्रण व्यूह-रचनाओं के निर्धारण में बहुत ही मददगार साबित हुआ है । परियोजना निदेशालय (पीडी-एडमास) ने पशुधन में अबोरशनकारी विभिन्न रोगों के आर्थिक प्रभाव विश्लेषणों के लिए स्प्रेड-शीट्स मोडयूल्स का भी अन्वेषण किया है ।

इस परियोजना निदेशालय (पीडी-एडमास) का मेरूदण्ड, महामारी यूनिट है, जिसके दो तरह के सेट है-स्थितिकी एवं गतिकी । रोग-प्रोफाइल डेटा में सम्मिलित है - प्रजातियों में जिला विशेषों में हमलों की संख्या, सुग्राहय पशु, मृत्यु-दर, प्रत्येक रोग का टीकाकरण एवं रोग प्रस्फोटों के महिने एवं वर्ष आदि । फिलहाल डेटा-बैंक में देश के 1987 से लेकर 2010 तक के विविध पशु-धन रोग प्रस्फोट रिपोर्टें के दस्तावेजों 77,777 के डेटा उपलब्ध हैं । इस बैंक के समुचित वैध्यता वाले गतिकी डेटा नियमित रूप से ननीनतम् (अपडेटिड) किए जाते रहें हैं । कथित डेटाओं के विश्लेषण से यह पाया गया कि एफएमडी, एचएस एवं बेबेसियोसिस देश के क्रमश: विषाणु, बैक्टीरियल एवं पैरासाइटिक पशु-रोग हैं । आर्थिक रूप से महत्व-पूर्ण रोगों, जैसे बीक्यू और एचएस के लिए, इकोपैथोजोन्स तैयार किए गए । नेडरेस (NADRES) मॉडल का प्रयोग करके आर्थिक रूप से 15 महत्व-पूर्ण रोगों में एक लोडिस्टिक रिग्रेशन विश्लेषण किया गया और 12 कलेण्डर महिनों के लिए विभिन्न रोगों के भविष्यवाणी नक्शे तैयार किए गए । आकाशीय भविष्यवाणी विज्ञान के अनुसार यह निष्कर्ष निकाला गया कि देश के लगभग सभी राज्यों में हीमोर्रहेजिक सेप्टिमिया वर्ष-पर्यन्त सबसे बडा रोग-प्रस्फोट है, जो कि बाद में ब्लैक-क्वार्टर का रूप धारण कर लेता है । एफएमडी, पीपीआर, थाइलीरिया, ट्राई-पैनासोमा एवं बेबेसिया और एक आंत्र-रोग (फेसियोला परजीवी) नामक स्पेटिला विश्लेषण पर अध्ययन किए गए । सामायिक महामारी विज्ञान में, आंचलिक स्तर पर पशुधन के कुछ प्रमुख रोगों पर अध्ययन किए गए और यह अध्ययन डेटा-बैंक में उपलब्ध, 2001 से 2010 तक के 10 वर्षों के डेटा पर आधारित था । अध्ययन में यह पाया गया कि 6 अंचलों की रिपोर्ट (उत्तरी, दक्षिण, पूर्वी, पश्चमी, केन्द्रीय और उत्तर-पूर्वी) में एचेस अवधि से ज्यादा पायी गयी, जबकि बीक्यू एवं एन्थ्रेक्स में संगत रूप से कोई रिपोर्ट नही पायी गई । परिणामों में यह भी पाया गया कि दक्षिण अंचल रिपोर्ट सतत रूप से अभिलेखित पायी गई, जबकि उत्तरी पूर्वी अंचल में ऋणात्मक अथवा न्यून-स्तरीय रिपोर्ट पायी गई । भैसों की अपेक्षा अन्य पशुओं में रोगों के अधिक अभिलेख पाये गए । इसी प्रकार से पीपीआर पर भी अध्ययन किए गए । परिणामों में, 2003 तक की सभी अंचल रिपोर्ट पायी गई । 2003 से बाद वाली अवधि की रिपोर्ट में रोग की सतत घटनाएं रिपोर्ट में पायी गई और अन्य अंचलों में नगण्य घटनाओं में शून्य रिपोर्ट पायी गई । ''प्रत्यक्ष लागतटट (परिणाम/नुकसान/संसाधन बरबादी, उपचार एवं निवारण लागत आदि के) आंकलन करने के दृष्टिकोण से आर्थिक प्रभाव विश्लेषणों के लिए प्रत्येक रोग के अलग-अलग स्प्रेड-शीट मोडयूल्स तैयार किये गए । प्रजनन समस्याएं वाले पशुओं में पोषण स्तरों के दृष्टिकोण से आमने सामने संगठित फार्मों के बोवाइनों मे पायी गई प्रजनन कुव्यवस्थाओं में आर्थिक प्रभावों ने जिंक के घटने हुए स्तरों को पाया गया ।

अपने देश में उपलब्ध अपने तरह की प्रथम निक्षेपस्थान वाली पद्धतियां, राष्ट्रीय पशुधन सीरम निक्षेपस्थान वाली पद्धतियां कहलाती हैं, जो महामारी जनसंख्या वाले पशुओं की आवश्यकताओं को पूरा करती हैं । इस परियोजना fदेशालय (पीडी-एडमास) की अन्य सहयोगिक यूनिटों द्वारा स्ट्रेटीफाइड रेन्डम सैम्पलिंग विधि से पांच पशुधन प्रजातियों (जानवर, भैंस, भेड, बकरी और सुअर) के सीरम नमूनों को संग्रह करके निक्षेपस्थान पर जमा कर दिया गया । वर्ष 2010-11 के दौरान, 08 पशुधन प्रजातियों के कुल मिलाकर 2466 मनूने संग्रह किए गए और पाई-डायाग्राम में उपलब्ध सीरम ब्रेक-अप को निक्षेपस्थान पर जमा कर दिया गया । अध्ययन के रूप में, इस परियोजना निदेशालय (पीडी-एडमास) को छंटाई के लिए अथवा ब्रूसेलोसिस की पुष्टि के लिए प्राप्त हुए, 1085 जानवरों के सीरम नमूने, एब-एलिसा AB-ELISA के थे और उनमें से 67% धनात्मक प्रतिरक्षियों वाले पाये गए ।

ब्रूसेलोसिस, पशुओं में पाया जाने वाला एक प्रजनन रोग है, जो मनुष्यों में भी देखने को मिलता है, जो दीर्घकालीन निशक्तता और बाझपन को जन्म देता है । पशुओं में एवं मनुष्यों में ब्रूसेलोसिस

के विभिन्न रोग निदान परीक्षणों /आस्सों को विकसित किया गया । विकसित किया गया अप्रत्यक्ष एलिसा को भारत के विभिन्न राज्यों की बकरियों में (एन=2362) एवं भेडों (एन=1702) प्रति ब्रूसेला-प्रतिरक्षियों को छांटने के लिए प्रयोग किया गया । उच्चतम सीरोप्रीवालेन्स क्रमश: मध्य-प्रदेश और बिहार की बकरियों में और कर्नाटक एवं राजस्थान की भेडों में पाया गया । अलगाव एवं पीसीआर विधि से भेडों और बकरियों के प्रतिनिधि धनात्मक एवं ऋणात्म रक्त एवं सीरम में आई-एलिसा (iELISA) का मूल्यांकन किया गया । आइ-एलिसा (iELISA) के लिए अनन्तिम पैटेन्ट संख्या-01592/CHE/2008 प्राप्त कर ली गई । स्वाइन में ब्रूसेला प्रतिरक्षियों की जांच के लिए एक अप्रत्यक्ष एलिसा को विकसित किया गया । ब्रूसेला अलगावों के निक्षेप-स्थान भारी मात्रा में पाए गए । मल्टीप्लेक्स ब्रूस लेड्डर एवं आमोस पीसीआर द्वारा ब्रूसेला अलगावों की प्रजातियों की पहचान की गई । ब्रूसेला अलगावों का फाइलोजेनेटिक वृक्ष - ओएमपी 2बी जीन आधारित श्रेणियों का विश्लेषण करके, तत्पश्चात आध्ययन किया गया । मनुष्यों एवं पशुओं में ब्रुसेलोसिस के निदान के लिए एक सामान्य रोग-निदानात्मक परीक्षण को विकसित किया गया, जिसे एक महत्वपूर्ण उपलब्धी कहा जा सकता है । मनुष्यों में ब्रूसेलोसिस के निदान के लिए, पुनर्योजक बीपी 26 (bp26) प्रोटीन आधारित एलिसा को विकसित किया गया, जिसे संस्थान की एक अतिरिक्त उपलब्धी कहा जा सकता है ।

पहुंच से बाहर के एक कार्यक्रम के तहत, प्राणियों में पाए जाने वाले रोगों पर ब्रूसेलोसिस की महामारी, लेप्टोस्पाइरोसिस एवं लिस्टिरियोसिस के अलावा भी अध्ययन किए गए । पशुधन में इन एजेन्टों का बोझ और उनके उत्पादों का बोझ पयाप्त रूप से पाया गया । सबसे बडी रूची की बात यह पायी गई कि सुअर नमूनों मे 51.2%, 74.35% एवं 20.51% नमूने ब्रूसेलोसिस के क्रमश: आरबीपीटी, एलिसा और पीसीआर के धनात्मक पाए गए, जोकि सुअर नमूनों में प्रतिजन एवं प्रतिरक्षियों दोनो में उच्चतम प्रीवालेन्स का संकेत देती हैं । विभिन्न पशुधन प्रजातियों में लेप्टोस्पाइरा के लिए दोनो पीसीआर एवं अलगाव विधियों द्वारा 11 चीते एवं अश्व सहित मनूनों की छंटनी की गई । 10 जानवरों मे से 04 जानवर और 16 बकरियों में 11 बकरियों के सीरा नमूने लिस्टीरिया मोनोसाइटोजन्स प्रजातियां विशिष्ट पीसीआर के धनात्मक पाए गए । जोखिम से भरे मनुष्यों (पशुओं से जुडे हुए पशु-चिकित्सकों, पैरा-पशु-चिकित्सकों, कृषकों एवं श्रमिकों और क्लिनिकल संकेत दर्शाने वाले मनुष्यों) से संग्रह किए नमूनों मे ब्रूसेलोसिस, लेप्टोस्पाइरोसिस एवं लिस्टिरियोसिस की प्राणी-रूपीय क्षमता का मूल्यांकन किया गया ।

आण्विक महामारी में पशुधन एवं पशुधन उत्पादों के रोगजनकाणुओं से युक्त भोजन में बहुत से महत्वपूर्ण परिणाम देखने को मिले । BURST विश्लेषण में यह पाया गया कि अलगावों के इस संग्रह में (एसटी-7 क्लोनल कम्प्लेक्स) एक मेजर लाइनेज थी, जिस में एसटी-7 के एकल लोकस बैरियान्ट के रूप मैं, एसटी-12 के साथ 34 एसटी पाया गया । वर्तमान आध्ययन में प्रयोग किया गया डेटा, एल. मोनोसाइटोजन्स से अवतरित किया गया है, जोकि जिन्स वितरण के फ्रमवर्क को आकाश प्रदान करता है, क्योंकि रोगजनकाणुता पर अधिक महत्व दिया जा सकता है और जिसे जनसंख्या संरचना को समझने एवं इस रोगजनकाणु की वैश्विक महामारी में सुधार लाने के लिए भविष्य में विस्तार भी किया जा सकता है । देश में लेप्टोसीरा अनुसंधान के क्षेत्र में, परियोजना निदेशालय (पीडी-एडमास) का अपना एक विशेष स्थान है । परियोजना निदेशालय (पीडी-एडमास) की स्थापना से लेकर आज तक, डाइवर्स पशुओं से लेकर मनुष्यों के ग्रुपों तक लेप्टोस्पाइरोसिस में अनुसंधान गितिविधियों के अन्तर्गत एक साधारण एक लेप्टोस्पाइरल स्टेनिंग किट को विकसित किए जाने ले लेप्टोस्पाइरा अलगावों को एक बारी संख्या में बल मिला है । अत: इस देश में समय-समय पर मनुष्यों एवं विशेषकर पशुधन में लेप्टोस्पाइरोसिस का संवीक्षण करने के लिए लेप्टोस्पाइरा अलगावों की पहचान एवं चरित्रांकन करना दुष्कर कार्य है । इस से देश में प्रभेद किए जाने की सुविधा एवं मैट (MAT) रोग-निदान विधि में प्रतिजनों के पैनल के चयन की सुविधा उपलब्ध हो जाएगी । सरोवार हार्डजो जैसी लेप्टोस्पाइरा प्रजातियों के पाणिरूप का अपना एक विशेष महत्व है । संक्रामक जानवरों को रखरखाव होस्ट्स के रूप में जाना जाता है, परिणाम स्वरूप यह बीमारी अबोर्शन, बांझपन और थनेले-रोग के रूप में सामने आती है और वे कमजोर अथवा क्लिनिकली सामान्य बछडों को अभी भी जन्म देते हैं । किन्तु सम्पर्क पशुओं एवं मनुष्यों में हारबर संक्रामण एक सशक्त स्त्रोत के रूप में देखने को मिलता है । rLigB/lgG जैसी लेप्टोस्पाइरा एसपीपी का प्रयोग करके इम्मयूनो आस्से का मानकीकरण किए जाने का कार्य प्रगति पर है, इस से पशु-ग्रुपों में रोगजनकाणुओं एवं नॉन-रोगजनकाणुओं वाले लेप्टोस्पाइरल संक्रमणों में अन्तर करना आसान हो जाएगा ।

संस्थान में, बोवाइन्स में ट्रीपानोसोमियासिस के कैरियर स्टेटस के रोग-निदान करने की सुविधा उपलब्ध हो और टी.इवान्सी के विरुद्ध प्रतिरक्षियों का पता लगाने के लिए पुनर्योजक वीएसजी प्रोटीन आधारित एलिसा को विकसित करने कार्य मानकीकरण के तहत है । कुछ हीमोप्रोटोजोन परजीवी संक्रमणों के ईको-पैथोजोन नक्शे भी सृजित किए गए हैं ।

(एनएआईपी प्रधान परियोजना के अन्तर्गत) रोगजनकाणुओं एवं उनसे सम्बन्धित थनेला रोग करकों की स्थानीय एवं वैश्विक महामारी के विशेष सन्दर्भों को लेकर, थनेला रोग की महामारी पर गहन एवं सार्थक अध्ययन किए जा रहे हैं । इस प्रकार के अध्ययन किए जाने के लिए संस्थान में सभी आवश्यक औजार भी उपलब्ध हैं । डेयरी फार्मों से संग्रह किए गए दूध के नमूनों में थनेला रोग के मेजर रोगजनकाणुओं के अलगाव एवं परिचय पाने के सन्दर्भ में प्रयोग किए गए । कॉलोनी मोर्फोलोजी पर आधारित कुल प्राप्त 85

दूध के नमूनों में 205 जीवों का अलगाव एवं रंग, आकृति एवं आकार आदि की अन्य सांस्कृतिक चरित्रांकन सम्बन्धित गतिविधियां सम्पन्न की गई। जीन्स एवं प्रजातियों द्वारा विशिष्ट पीसीआर/एमपीसीआर PCR/mPCR आस्से का मानकीकरण का कार्य पहले ही सम्पन्न किया जा चुका है। पाए गए सबसे अधिक पूर्व-प्रभावकारी रोगजनकाणुओं में स्टेफ़ायोकोकस एसपीपी (71.70%), स्ट्रेप्टोकोकस एसपीपी (14.15%) और ई.कोली (14.15%) शामिल हैं। स्टेफ़ायोकोकस के आण्विक महामारी विज्ञान से यह पता चला कि S.aureus का कोई भी अलगाव ST 398, प्राणीरूपीय महत्वपूर्ण कारक है। थनेला रोग के रोगजनकाणुओं की प्रीवालेन्स और इसके एन्टीबायोटिक/मेथीसिलिन अवरोध अध्ययनों के बीच पाया जाने वाला सह-सम्बन्ध, उपक्लिकल थनेला रोग के निदान में बहुत लाभकारी सिद्ध हो सकेगा। यह एक ऐसी उपलब्धी है जिसमें 14-थनेला रोग के रोगजनकाणुओं का पता लगाने में, मात्रात्मक मल्टीप्लेक्स पीसीआर का मानकीकरण किया गया।

PPRV प्रतिरक्षियों के लिए, PPR C-ELISA किट का प्रयोग करके, कुल मिला कर 2609 सीरम नमूनें (बकरी-92, भेड़-356, जानवर के 2162) स्क्रीन किए गए। बकरी, भेड, और जानवर का प्रीवालेन्स प्रतिशत क्रमश: 23.31%, 9.78% एवं 4.79% पाया गया और संस्थान के एक अन्य दूसरे सहयोगी संस्थान द्वारा, PPRV प्रतिरक्षियों के लिए, PPR C-ELISA किट का प्रयोग करके, कुल मिला कर 660 सीरम नमूने (बकरी-265, भेड-317, बोवाइन एवं अन्य-78) स्क्रीन किए गए। बकरी, भेड़ और बोवाइन एवं अ्य का प्रीवालेन्स प्रतिशत क्रमश: 43.3%, 53.9% और 26.9% पाया गया। PPRV प्रतिजन के लिए, PPR C-ELISA किट का प्रयोग करके, कुल 268 क्लिनिकल नमूने (बकरी-190, भेड-75, बोवाइन एवं अन्य-02) स्क्रीन किए गए। बकरी और भेड का प्रीवालेन्स प्रतिशत क्रमश: 26.3% और 44% पाया गया। जानवर में पीपीआर का सीरोप्रीवालेन्स, बेसल-स्तर का है। PPR प्रतिरक्षियों के लिए, जानवर और भैंस के प्रीवालेन्स प्रतिशत में कोई विशेष अन्तर देखने को नही मिला, जबकि भेड और बकरी के बीच के बीच में उच्च स्तर का प्रीवालेन्स प्रतिशत देखने में आया।

ब्लूटंग पर महामारी विज्ञान का विस्तृत कार्य (एआईएनपीबीटी के भाग के रूप में) वर्ष 2009 में शुरु किया गया। परिजोजना निदेशालय (पीडी-एडमास) को सन्देहात्मक घाव प्रस्फोटों की सूचना सबसे पहले बीदर, राजचूर और गडग जिलों से प्रप्त हुई थी। कर्नाटक राज्य में पिछले वर्षो की अपेक्षा, ब्लूटंग पर घाव प्रस्फोटों की मार्जीनल कमी का संकेत देने वाले अभिलेख प्राप्त हुए हैं। रूचि की बात यह है कि प्रभावित पशुओं की संख्या पिछले वर्षों की अपेक्षा आधी से थोडी कम है और ऐसे ही अभिलेख रोग के कारण हुई पशु-मृत्यु के विषय में भी पाए गए हैं। इस से स्ट्रैन /सीरो-टाइप/फ्लॉक इम्म्यूनिटी से सम्बन्धित कार्य करने की आवश्यकता पर जोर दिया गया है। GPS और GIS सम्बन्धित महामारी फैलाने वाले कारके BT के स्थानीयकरण में सहायक सिद्ध हो सकेगा। प्राइमरी विश्लेषण से यह पता चला कि बागलकोट जिले से संग्रह किए गए क्लिनिकल नमूने सीरो-टाइप-1 के धनात्मक थे और सिन्धनूर जिले से संग्रह किए गए क्लिनिकल नमूने सीरो-टाइप-2 एवं सीरो-टाइप-1 के धनात्मक थे। 677 सीरम नमूनों के विश्लेषण से यह पाया गया कि 185 नमूने (27.32%) प्रति BTV प्रतिशक्षियों के धनात्मक थे। स्वदेश में विकसित किए गए कीटाणु-जाल का प्रयोग करके BTV एवं कुलीकॉयड एसपीपी के बीच में सह-सम्बन्धों पर भी अध्ययन किए जा रहें हैं।

1995-2010 के दौरान, IBR की सीरो-महामारी से सम्बन्धित देश के विभिन्न भागों से संग्रह किए गए 57009 सीरम नमूनों का एब-एलिसा विधि द्वारा IBR प्रतिरक्षियों के लिए परीक्षण किया गया। जिनमें से 20749 नमूने धनात्मक (36% प्रीवालेन्स) पाए गए। IBR की समग्र प्रीवालेन्स में पाया गया अन्तर, नमूनों-आकार से सम्बन्धित हो सकता है। इस वर्ष के दौरान BoHV-1 संक्रमण की आण्विक महामारी से सम्बन्धित, जानवर/भैंस से संग्रह किए गए IBR/IPV के आठ क्लिनिकल नमूनों और तीन अन्य दूसरे अलगावों (एडमास-1, एसमास/258-08 एवं ऊटी) का अपने निक्षेप-स्थान पर विषाणु डिएनए उद्धरण, पीसीआर एवं gB जीन की आंशिक न्यूक्लियोटाइड श्रेणीकरण के विषय में परीक्षण किया गया। सुकृत एनटी श्रेणियों के तुलनात्मक अध्ययन से यह पाया गया कि सभी आल्फ़ा हर्पिस विषाणुओं में उच्च डिग्री की अपनी एक पहचान है। सभी भारतीय स्ट्रैन्स में न्यूक्लियोटाइड श्रेणी पहचान 99% से 99.5% के बीच में पायी गई। ऐसी ही कूपर स्ट्रैन के साथ पाया गया। पशुप्रजातियों और भारत में मेजर प्रीवलेन्स ग्रुप की क्लिनिकल अभिव्यक्तियों निर्पेक्ष में, इस अध्ययन में प्रयोग किए गए सभी भारतीय स्ट्रैन्स, उपटाइप 1.1 के थे। फाइलोजेनेटिक विश्लेषण, कुल मिलाकर, यह संकेत देता है कि BoHV1.1, भारत में पूर्व-प्रभावकारी उपटाइप प्रीवालेन्स का था।

इस वर्ष के दौरान, वाणिज्य किट का प्रयोग करके ब्लॉकिंग एलिसा पर आधारित, परम्परागत स्वाइन ज्वर की सुपर-प्रीवालेन्स पर परीक्षण किया गया और चार राज्यों (आन्ध्र प्रदेश, कर्नाटक, केरल, और महाराष्ट्र) से 1257 सीरम नमूनों का संग्रह किया गया। यह सांक्ष्य मिलता है कि 237 सीरम नमूने CSF प्रतिरक्षियों के धनात्मक थे। समग्र बाहय प्रतिथस धनात्मक - 19 था। आन्ध्र-प्रदेश में CSF प्रतिरक्षियों के प्रीवालेन्स के उच्चतम प्रतिशत के सांक्ष्य प्राप्त हुए हैं। जब कि केरल में (22%), महाराष्ट्र में (14%) और कर्नाटक में (12%) के साक्ष्य मिले हैं।

अन्त में यह निष्कर्ष निकलता है कि देश के पशु-स्वास्थ्य को बनाए रखने के लिए, परियोजना निदेशालय (पीडी-एडमास), राष्ट्रीय पशुधन रोग संवीक्षण एवं निगरानी के क्षेत्र में महत्वपूर्ण भूमिका निभा आ रहा है।

About PD_ADMAS

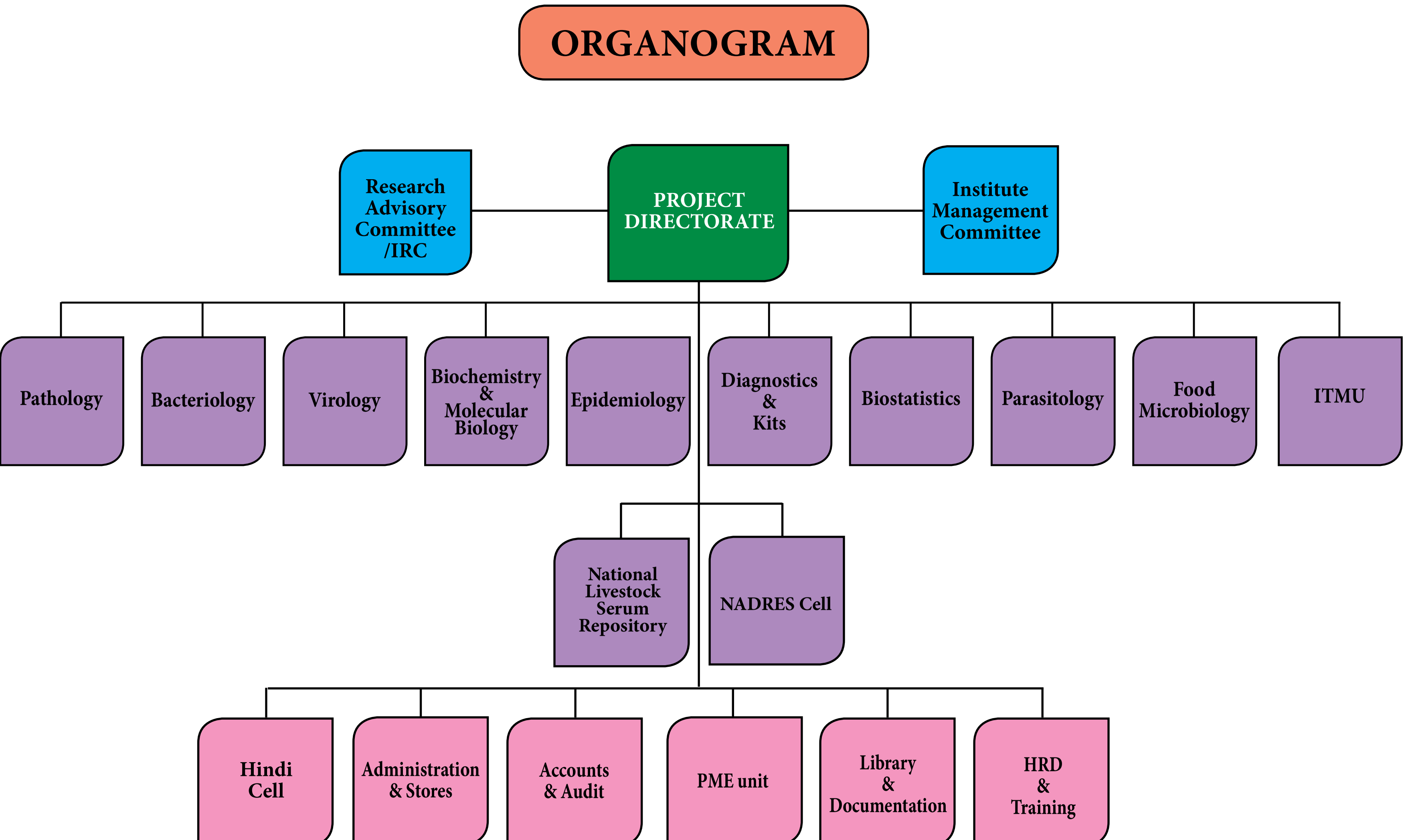
The project on animal disease monitoring and surveillance, which was initiated by the ICAR in the 7th five year plan as an All India Coordinated Research Project (AICRP) became fully functional during the last quarter of 1987 with the establishment of four Regional Research Units (RRUs) located at Bengaluru, Hyderabad, Pune, and Ludhiana. The Central Coordinating Unit (CCU) was established at the Institute of Animal Health and Veterinary Biologicals, Bengaluru to coordinate research activities of the regional units. ADMAS was further strengthened in the 8th plan with support of ICAR and European union by giving the responsibility of the National Project on Rinderpest Eradication (NPRE) involving the participation of 32 state level diagnostic/disease investigation laboratories. Later, realizing the impact of animal disease monitoring and surveillance on our entire livestock sector and to give a boost, ICAR upgraded this project to an independent institute status on 1st April, 2000 (during the IX plan) as – "Project Directorate on Animal Disease Monitoring and Surveillance (PD_ADMAS)" with ten collaborating units. The Directorate got further impetus with addition of five more collaborating units in the 10th plan. In XI plan Guwahati Centre in Assam has been included as a collabrating unit of AICRP, ADMAS.

Research mandates of PD_ADMAS

- Research and development on Epidemiology of livestock diseases.
- Understanding specific disease process for rational development of diagnostics and strategic control technologies for livestock diseases including zoonosis.
- Biodiversity of pathogenic microbes.
- Development of systems for forecasting and forewarning of economically important livestock diseases.
- Economics of livestock diseases and health care measures.

Research Mandates of Regional Research / Collaborating Units

- Sero-monitoring for important livestock diseases based on sample frame.
- Investigation of endemic, emerging and reemerging livestock disease outbreaks in respective area using innovative technologies.
- Participation/strengthening of National Livestock Serum Bank.
- Participation in strengthening of microbial pathogen repository at PD_ADMAS.
- Effective updating of NADRES with active disease and related meteorological data.
- Utilization of forecasting models through NADRES for forecasting and forewarning of livestock diseases.
- Collaborative study on economic losses due to livestock diseases and their control measures.


ORGANOGRAM
Research Advisory Committee /IRC
PROJECT DIRECTORATE
Institute Management Committee
Pathology
Bacteriology
Virology
Biochemistry & Molecular Biology
Epidemiology
Diagnostics & Kits
Biostatistics
Parasitology
Food Microbiology
ITMU
National Livestock Serum Repository
NADRES Cell
Hindi Cell
Administration & Stores
Accounts & Audit
PME unit
Library & Documentation
HRD & Training

Institute Research Projects

Project: 1

The epidemiology and forecasting of economically important livestock diseases of India

M.R. Gajendragad

The epidemiology unit of the Directorate has two sets of data, Static and Dynamic. The static data is the one which changes over a long period, eg for such data is land utility, crop production profile, agro-ecological profile, etc. whereas the dynamic data comprises of the meteorological profile, the livestock disease profile, demography, livestock population profile, etc. The directorate collects as well as compiles the data from various sources. The compilation of these data has resulted in the following databases which are being regularly updated and maintained.

1. Livestock disease profile
2. Demography
3. Livestock population profile
4. Land utility and crop production profile
5. Agro-ecological profile
6. Meteorological profile

Livestock disease profile

The disease profile data has been arranged with the linkage to zonal, state and district codes assigned to each zone, state and district, respectively. The data consists of number of attacks, susceptible animals, deaths and vaccinations with respect to each disease in a particular district with species of the animals involved, year and month of the outbreak. At present the data bank has a well documented 77,777 data pertaining to various livestock disease outbreak reports of the country from 1987 to 2010. The dynamic data in the databank is updated regularly after suitable validation.

The compiled data has been analysed as national disease profile, state disease profile and district disease profile. Based on these analyses the diseases have been ranked on their occurrence at national level. On analysis of the data it was noticed that FMD, HS, and babesiosis are respectively the top viral, bacterial and parasitic diseases reported from the country. The disease ranking as per the etiology has been depicted in Figs. 1, 2, and 3.

Based on the frequency of the occurrence of the outbreaks, the pathozones were calculated. The

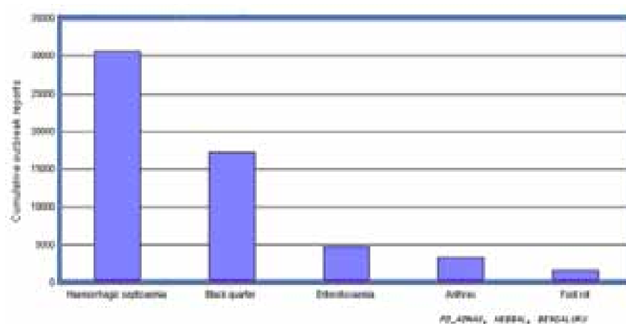


Fig. 1. Ranking of Bacterial Disease incidence based on cumulative OB reports (1987 – 2010)

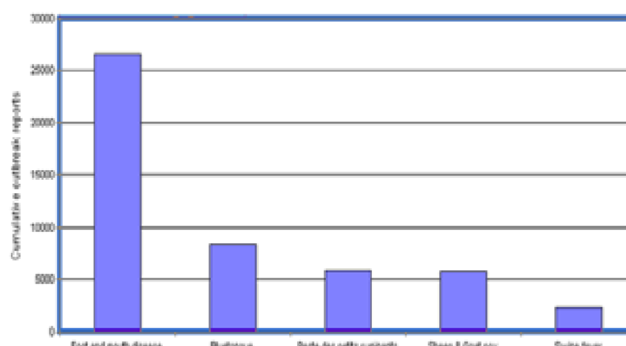


Fig. 2. Ranking of Viral Disease incidence based on cumulative OB reports (1987 – 2010)

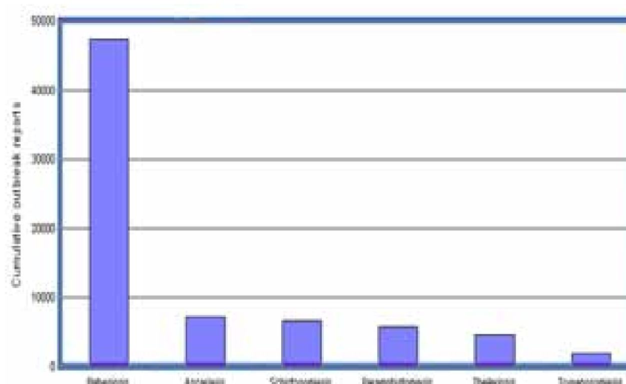


Fig. 3. Ranking of Parasitic Disease incidence based on cumulative OB reports (1987 – 2010)

correlation of the pathozones with the risk factors was carried to arrive at the ecopathozones. Thus the ecopathozones for economically important diseases have been prepared. Ecopathozones for BQ and HS are depicted in Fig. 4 and 5.

Animal Disease Forecasting

The latest data collected has been incorporated in the NADRES model and integrated to each district for each calendar month for a specific disease. A logistic regression analysis for 15 economically important livestock diseases has been carried out. The model

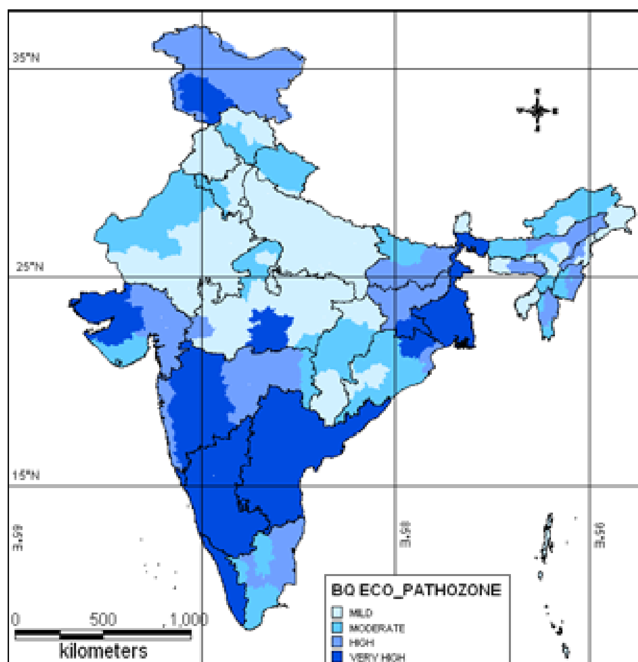


Fig. 4. Showing BQ ecopathozones

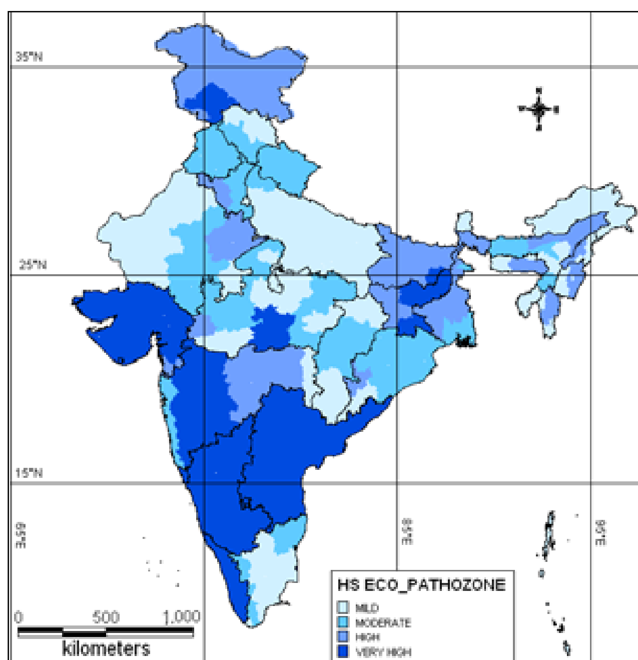


Fig. 5. Showing HS ecopathozones

predicts the probability of occurrence of the disease two months in advance in any particular district of the country. Based on the predicted group values obtained in the logistic regression model, forecast maps were prepared for the 12 calendar months. As an example, the south zone forecast map for HS has been shown at Fig. 6 and National forecast for BQ has been shown in Fig. 7.

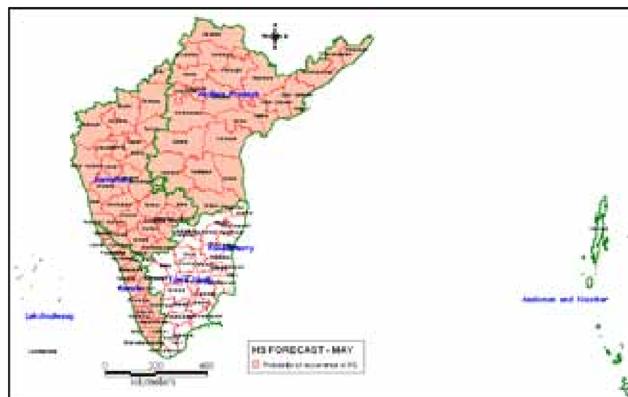


Fig. 6. South zone HS forecast map.

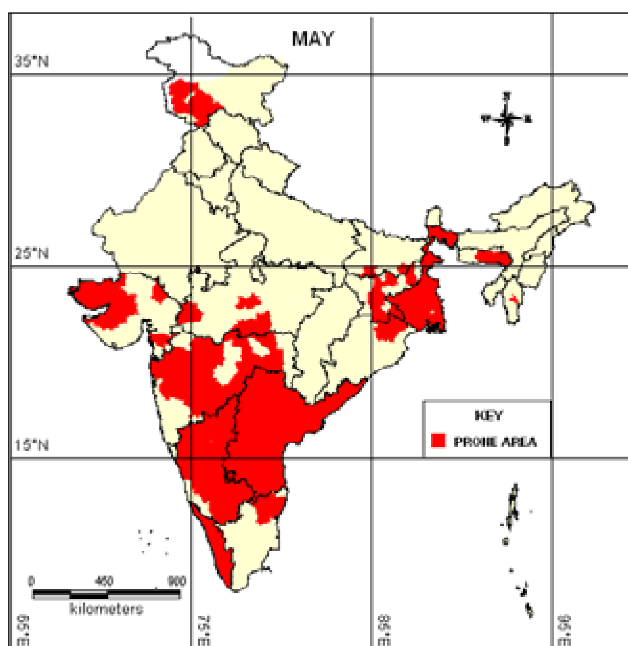


Fig. 7. National BQ forecast map.

Spatial Epidemiology of livestock diseases during the last decade

Bacterial diseases

Amongst the bacterial diseases haemorrhagic septicaemia tops the outbreak reports. It has been reported from almost all the states, throughout the year. Karnataka, Andhra Pradesh, Gujarat and Rajasthan have reported maximum number of cases. The big state like Uttar Pradesh showing a blank indicates that there is no data from that state. Similarly, black quarter has also been reported by all the states except Uttar Pradesh. Anthrax has been reported by all the southern states whereas in north, Punjab, Haryana and Uttaranchal have not reported. The study has

shown the endemicity of these diseases in the country with their occurrence consistently throughout the last decade (Figs. 8-11).

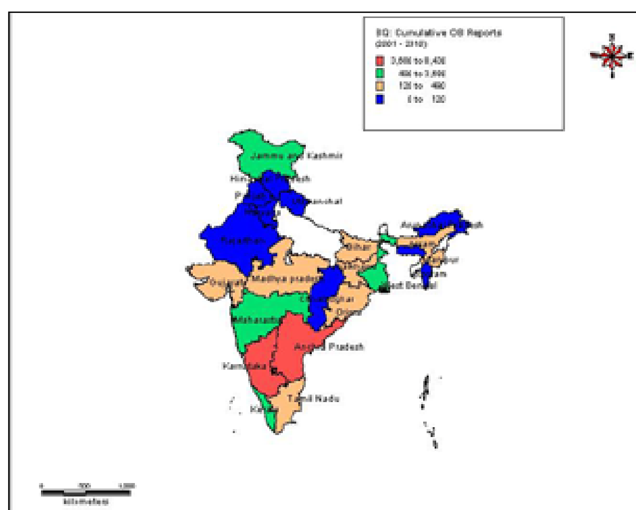


Fig. 8. Spatial distribution of BQ

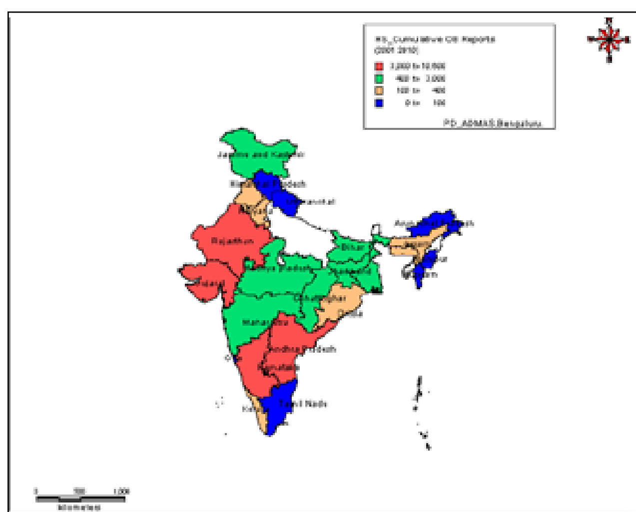


Fig. 9. Spatial distribution of HS

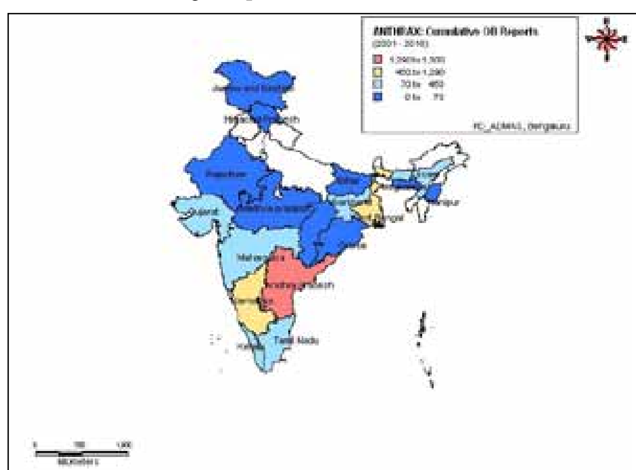


Fig. 10. Spatial distribution of Anthrax

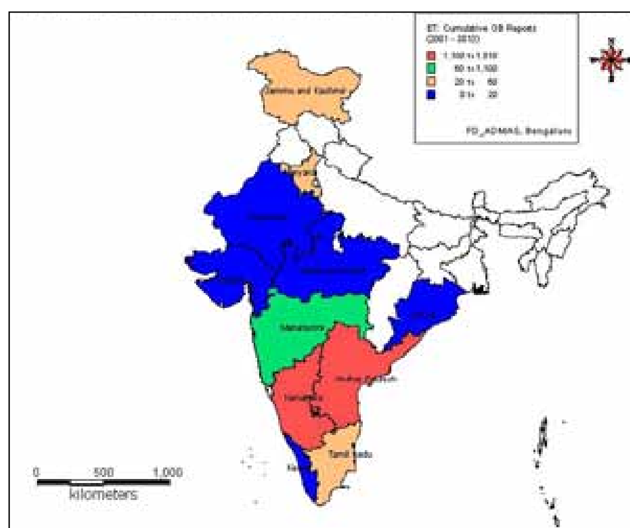


Fig. 11. Spatial distribution of ET

Viral Diseases

Three major viral diseases (FMD, PPR and Sheep & Goat Pox) were taken up for the study. The FMD has been more frequently reported from Karnataka, followed by AP, Kerala and WB with least reporting from Punjab, Haryana, HP, Rajasthan and Uttarakhand. Rest of the country showed moderate reporting in the range of 200 to 1200 OBs from 2001 to 2010. AP tops the list for PPR with >2000 OB reports followed by Karnataka and WB. It has been less reported from MP, Rajasthan, Punjab, Haryana, HP and Uttaranchal may be due to sparse sheep and goat population (Figs. 12-14).

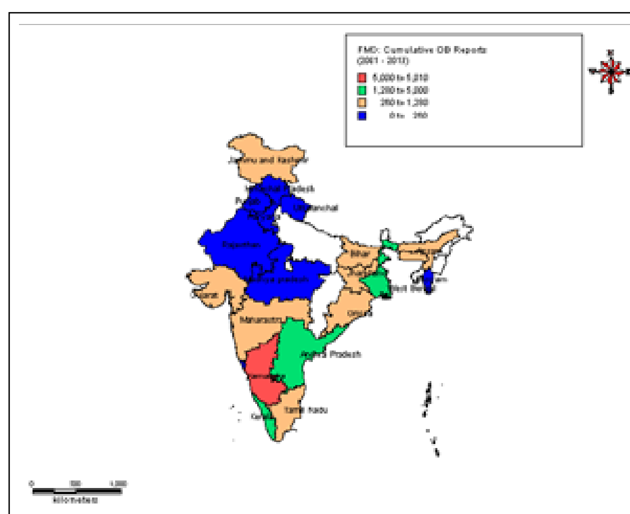


Fig. 12. Spatial distribution of FMD

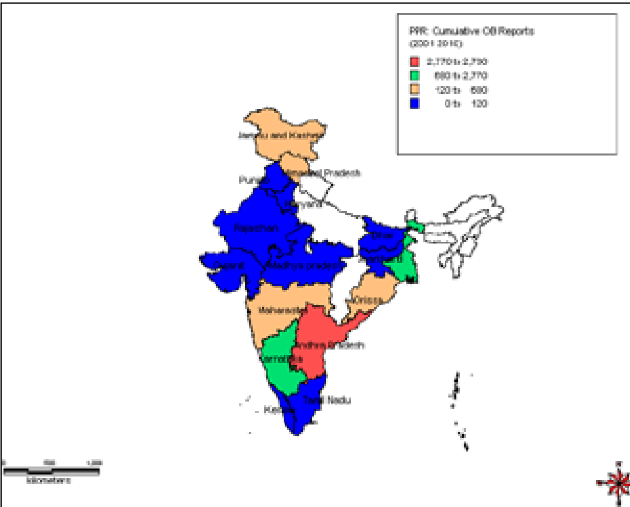


Fig. 13. Spatial distribution of PPR

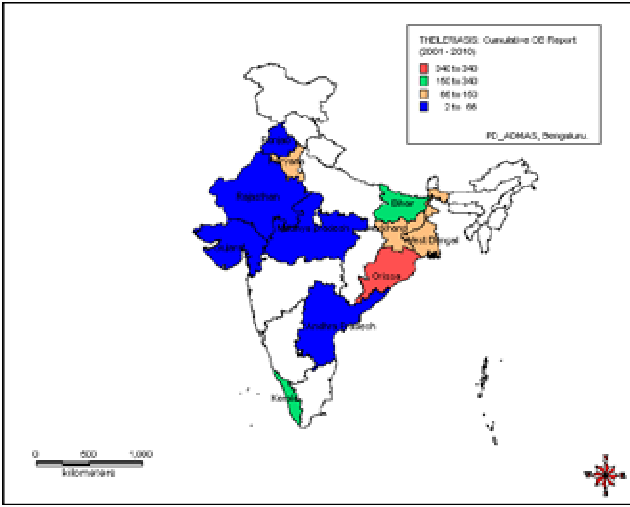


Fig. 15. Spatial distribution of Theileriasis

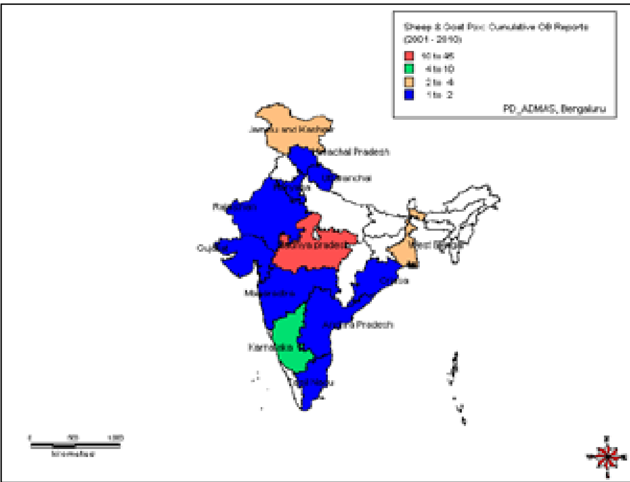


Fig. 14. Spatial distribution of Sheep and Goat Pox

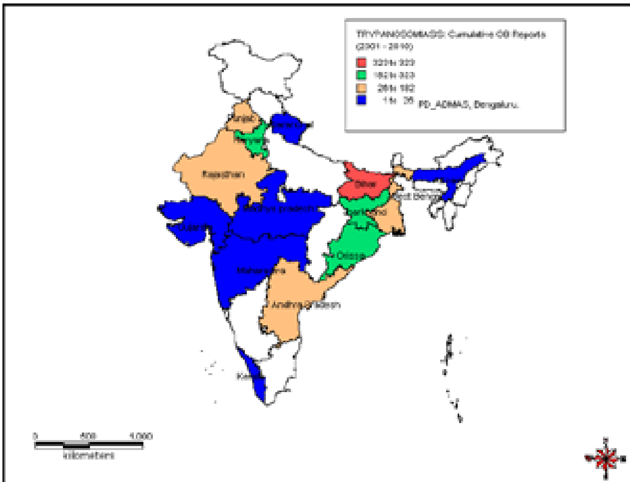


Fig.16. Spatial distribution of Trypanosomiasis

Parasitic diseases

Since parasitic diseases do not assume the proportion of outbreaks as is the case with viral or bacterial diseases, their reporting is poor. However, based on the reported cases, the study was taken up on spatial analysis of three major haemoprotozoan (*Theileria* sp, *Trypanasoma* sp and *Babesia* sp) and one gastrointestinal (Fasciola) parasites. There are more reports of these parasites from northern India than in south. The presence of trypanosomiasis in north India could be due to more of buffalo population than in south India. However no concrete conclusions can be drawn for want of data. (Figs. 15-18)

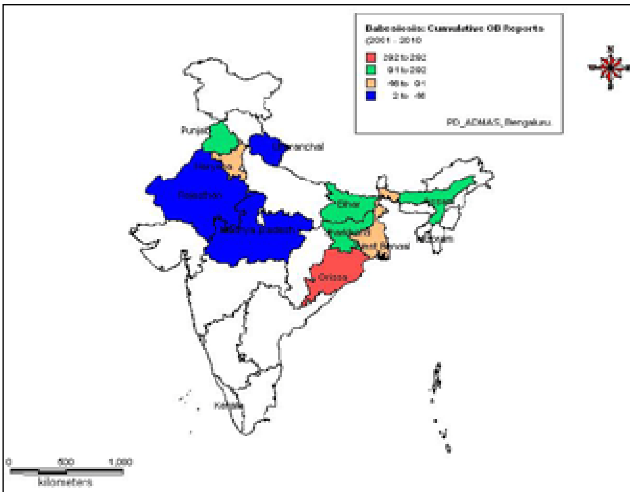


Fig. 17. Spatial distribution of Babesiosis

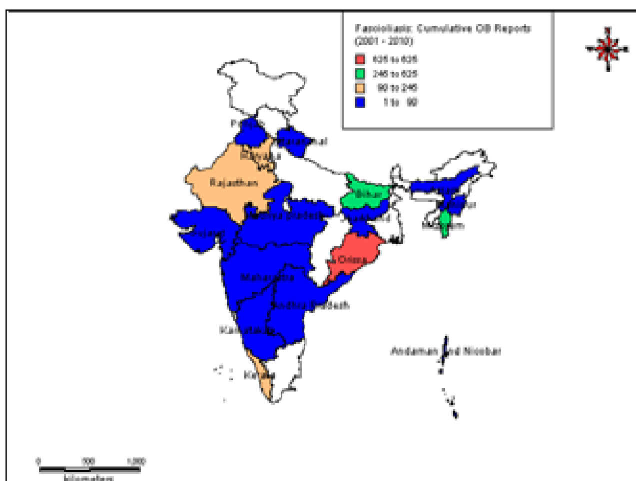


Fig. 18. Spatial distribution of fascioliasis

Temporal Epidemiology of livestock diseases during the last decade

Livestock Disease Trend during the last decade

During the period under report the trend of some of the major livestock diseases was studied at zonal level. The study is based on ten years (2001 to 2010) data available in the databank. It was noticed that all the six zones (North, South, East, West, Central and North-East) report HS over the period whereas for BQ and anthrax there is relatively no reports. The results also indicate that in the south zone the disease has been consistently recorded whereas in north-eastern zone there is low or negligible reporting of the disease The reason could be that the diseases are being diagnosed and reported more promptly in the south zone states and it is not so in other zones (Fig. 19). The disease as reported by various states has been shown in Fig 20. Only those states where the disease is more consistently reported are included in the graph. The disease has been more recorded in cattle than in buffaloes (Fig. 21). Further, it may be noticed that there is no lean period for the disease occurrence. The month of occurrence of the disease has been studied with respect to various districts of Karnataka also showed that the disease occurs irrespective of month although August records the highest reports (Fig. 22).

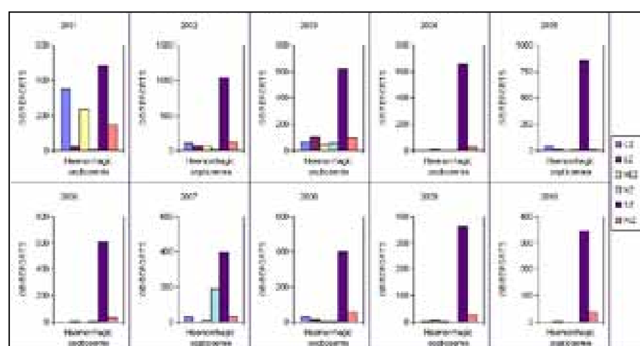


Fig. 19. Zone-wise HS trend during 2001 to 2010

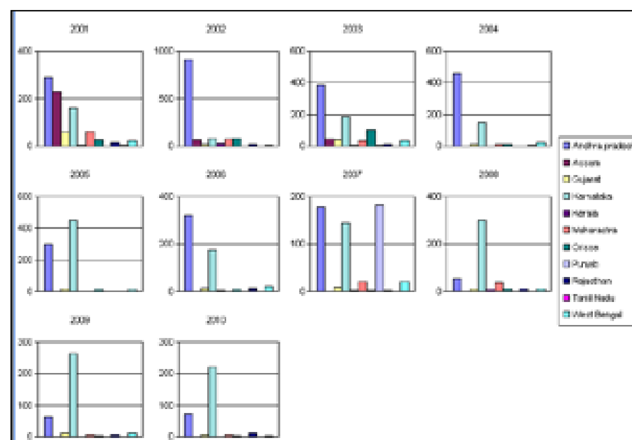


Fig. 20. Trend of HS outbreak

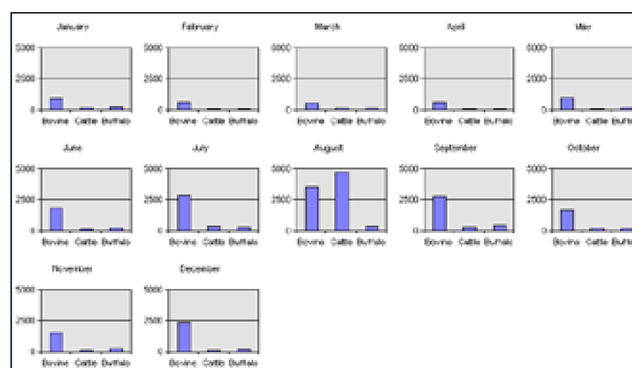


Fig. 21. HS in Bovine population

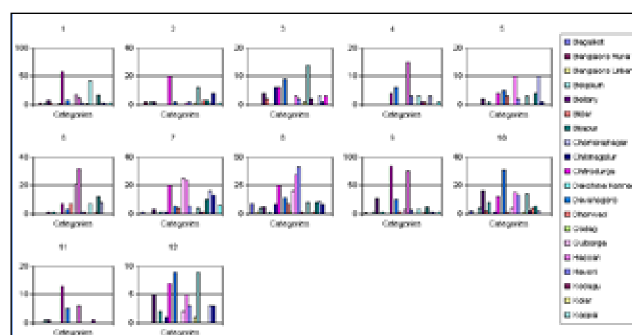


Fig. 22. HS reports from various districts of Karnataka with respect to months

Similar studies conducted on PPR showed that initially all the zones reported the disease up to the year 2003 and later on the reports dwindled to the extent that by 2010 only SZ and WZ reported the disease (Fig. 23). The results also indicate that there

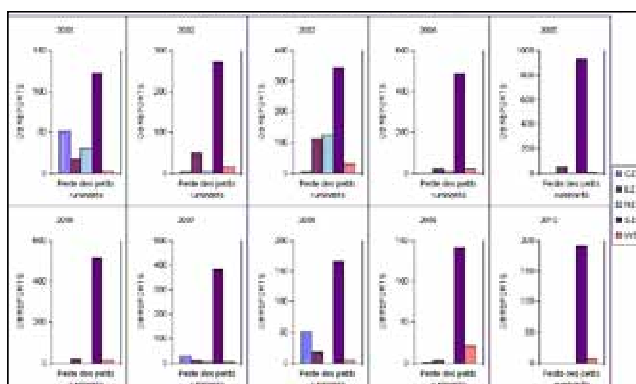


Fig. 23. Zone-wise PPR trend during 2001 to 2010

is an urgent need for in depth study for such decline. A similar trend has been noticed in case of BQ. The SZ showed a consistent disease incidence and the other zones showed nil to negligible incidences. This could be a result of under reporting. The temporal analysis clearly showed that there is gross under reporting which is highly visible in certain zones and calls for a national policy to bring out a drastic modification in the disease reporting system.

Project: 2

Development of spreadsheet modules for economic impact analysis

M.R. Gajendragad

The livestock diseases pose devastating economic losses to livestock farmers and the agricultural sector as well as animal and human health threats in the case of zoonoses. A research project has been taken up to provide assessment of economic impact of some of the nationally important livestock diseases in the form of spreadsheet models. Such an assessment is required to support policy decisions concerning livestock disease control and research priorities. The models will be used to estimate the 'direct costs' (output loss/ resource wastage, treatment and prevention costs) of each disease. The values of key disease variables within the models are determined from the scientific literature and by means of a survey of experts for each disease. 'Border prices' will used to value outputs rather than market prices. 'Low', 'medium' and 'high' ranges of key variables are used within the models to reflect variations in estimates. A spreadsheet module for FMD has been worked out based on the scientific literature. The PD_ADMAS data is being incorporated for the module. Its workability and validation is being taken up.

Project: 3

Economics of reproductive disorders in bovines of organized farms vis-à-vis to nutritional status

P. Krishnamoorthy, M.R. Gajendragad, K. Prabhudas, J. P. Ravindra, Raghavendra Bhatt and D.T. Pal

Sample survey of organized dairy farms was carried out and selected farms in Hubli, Dharwad, Bijapur in Karnataka and Pondicherry, Chennai in Tamilnadu for the study. Paired serum samples were collected from 128, 72, 123, 109 and 138 cattle in Hubli, Dharwad, Bijapur, Pondicherry and Chennai, respectively. Reproductive history like repeat breeding, abortion, metritis, retention of placenta, pregnancy and milk yield data were collected from the organized farms. Feed and soil samples were also collected. Out of 570 serum samples screened for Brucella and IBR antibodies, 254 (44%) and 158 (27%) were found positive, respectively. Serum copper and zinc levels were estimated and found zinc levels decreased in animals with reproductive problems. Feed analysis revealed that the crude protein, crude fat and metabolizable energy in concentrates and fodder fed to cattle were within the normal range. Soil analysis for mineral levels varied widely depending on the type of the soil in the organized farms. Serum and feed samples from Hubli, Dharwad, Bijapur, Pondicherry and Chennai farms were submitted for hormone and mineral analysis at NIANP, Bengaluru. Economic analysis of the data collected from the farms is being carried out. Results are awaited.

Project: 4

Studies on the epidemiology of the Peste des petits ruminants (PPR) in India

V. Balamurugan, P. Krishnamoorthy, M.R.Gajendragad, K.Prabhudas, Arnab Sen, V. Bhanuprakash and K.K. Rajak

A systematic study was undertaken on the epidemiology of PPR in sheep, goats and cattle including wild ruminants and the project was proposed during October 2009 with an objective of

generating the baseline data on prevalence of the PPR in India especially on sero-epidemiology or clinical prevalence of disease in collaboration with Indian Veterinary Research Institute, Mukteswar. The generated information will be very useful for effective disease management and in the development of a PPR control programme using indigenously developed PPR virus (PPRV) vaccines. The approved project was started during November 2009 and in continuation of the early year, the research work carried out during the period under report is described below.

A total of 2609 serum samples were collected from unvaccinated sheep, goats and cattle from different places of southern India, to study the sero-prevalence of PPR. The clinical samples were also collected from the unhealthy sheep, goat and cattle showing the respiratory symptoms such as nasal and ocular discharges to study the clinical prevalence in livestock. The collected serum samples were analyzed for PPRV specific antibody using the indigenous PPR competitive ELISA (cELISA) kit and the PPRV antigen in the infected swab materials was detected by using PPR Sandwich ELISA (sELISA) kit (kits were procured from IVRI, Mukteswar). None of the clinical samples were found positive for PPRV antigen, as tested by PPR S-ELISA kit. A total of 2609 serum samples (Goat-92, Sheep-356, Cattle-2161) were screened for PPRV antibodies by using PPR c-ELISA kit, the percentage prevalence of 23.31, 9.78 and 4.79, were observed in sheep, goat and cattle, respectively (Table 1).

At IVRI, Mukteswar, serum and clinical samples from different geographical locations of the India obtained through various organizations including organized goat/sheep farms, state animal husbandry laboratories, research institute and samples from small-herd owners submitted to the laboratory for PPR diagnosis were analysed for PPRV specific antibody using the PPR cELISA kit and the PPRV antigen in the infected swab materials/post-mortem samples was detected by using PPR sELISA kit. A total of 660 serum samples (Goat-265; Sheep-317; Bovine/others-78) were screened for PPRV antibodies by using PPR cELISA kit, the percentage positivity 43.3, 53.9 and 26.9 were observed in goat, sheep, and bovine respectively (Table 2). A total of 268 clinical samples (Goat-190; Sheep-75; Bovine/others-2) were screened for PPRV antigen by using PPR sELISA kit, the percentage positivity 26.3 and 44 were observed in goat and sheep, respectively (Table 3).

In overall, the present study describes sero-surveillance of PPR in livestock carried out during the period report using the serum samples randomly collected from different parts of India. In general, the per cent positivity of the antibodies in sheep and goats indicates enzooticity of the disease in the country, which is attributed to variations in the sheep and goat husbandry practices within different geographical regions, the agro-climatic conditions, the topography of different states, the socio-economic status of individual farmers and the migration of livestock in India. This report also presents the results of PPRV specific antibodies in situations where the sub-clinical infection was suspected in cattle and buffaloes. A total of 2609 serum samples from cattle and buffaloes, 356 from sheep and 92 from goat, which were associated with bovine population were collected from different states of southern India and were screened for PPRV antibody by using indigenously developed PPR monoclonal antibody-based cELISA kit. Serum samples were screened for PPRV antibodies and their per cent positivity with prevalence rate are presented in Table 1 as well as depicted in map and graphs (Figs. 24-26).

Based on the screening of the 2609 serum samples, the mean ± standard error (SE) per cent positive were 4.58±0.08, 23.31±0.99 and 4.76±0.49 in bovine, sheep and goat, respectively. The apparent prevalence of PPRV antibody in bovine, sheep and goats were 4.58% [95% confidence interval (CI): 4.52 -4.74)] and 23.31% [95% CI: 21.35-25.26] and 9.78% [95% CI: 3.79-5.73] respectively with overall prevalence of 7.61% [95% CI: 7.42-7.97], during the period report (Table 1). The true prevalence of PPRV antibodies in cattle, buffalo, sheep and goat were 5.24, 4.85, 25.69 and 5.26 respectively. Based on the analysis of the 2161 serum samples from bovine population in southern peninsular India, the overall percentage prevalence of antibody in Karnataka, Tamilnadu, Puduchery and Andhra Pradesh were 4.22, 9.65, 4.32, and 1.3, respectively (Fig. 24). High per cent prevalence of PPR antibodies (above 10%) in cattle from Chennai and Ramanagaram (Table.1) was observed, which may be due to the co-existence of infected sheep and goat population as reported earlier as PPRV infection needs close contact between infected and susceptible animals to spread. High prevalence of antibodies against PPRV in cattle has also been reported. Earlier study showed, high sero-prevalence rate of 67.42% in buffalo and

Table 1. Prevalence of PPRV antibodies in southern peninsular India

State	Place	Cattle Cows	Positive	Sheep	Positive	Goat	Positive	Total	Positive
Tamilnadu	Chennai farm 1	131	11 (8.40)	-	-	-	-	131	11(8.40)
	Chennai farm 2	128	14 (10.94)	-	-	18	2 (11.11)	146	16(10.96)
Andhra Pradesh	Tirupati farm 1	130	3 (2.31)	-	-	-	-	130	3(2.31)
	Tirupati farm 2	100	-	2	-	-	-	102	-
Karnataka	Ramangaram	19	2(10.52)	26	17 (65.38)	68	6 (8.82)	113	25(22.13)
	Devenahalli	1	-	-	-	-	-	1	-
	Viswanathapura	1	-	16	3 (18.75)	6	1 (16.67)	23	4(17.39)
	Kagglipura	60	3(5.00)	59	27 (45.76)	-	-	119	30(25.21)
	Bengaluru farm 1	150	3(2.00)	70	19 (27.14)	-	-	220	22(10.00)
Puducherry	Puducherry	440	19(4.32)	-	-	-	-	440	19(4.32)
		1160	**55 (4.74)**						
		Buffalo							
Karnataka	Dharwad	212	5 (2.36)	-	-	-	-	212	5(2.36)
	Bijapur farm 1	309	14(4.53)	-	-	-	-	309	14(4.53)
	Bijapur farm 2	240	15(6.25)	-	-	-	-	240	15(6.25)
	Bijapur farm 3	240	10(4.17)	-	-	-	-	240	10(4.17)
		1001	**44 (4.39)**	-	-	-	-	-	-
Karnataka	Bengaluru farm 2	-	-	45	5 (11.11)	-	-	45	5(11.11)
	Doddabalapura	-	-	101	1 (0.99)	-	-	101	1(0.99)
	Siddalghatta	-	-	37	11 (29.73)	-	-	37	11(29.73)
	Total	**2161**	**99(4.58)**	**356**	**83(23.31)**	**92**	**9(9.78)**	**2609**	**191(7.61)**
	Mean ± SE	-	**4.58 ± 0.08**	-	**23.31 ± 0.99**	-	**4.76 ± 0.49**	-	**7.61 ± 0.18**
	CI at 95% level	-	**4.42 - 4.74**	-	**21.35 - 25.26**	-	**3.79 - 5.73**	-	**7.42 - 7.97**

Figures in parenthesis indicate percentage

41.86% in cattle with significant difference (P=0.005) was reported using small sample size of cattle (n=43) and buffalo (n=89) sera.

Further, the percentage inhibition (PI) values obtained in c-ELISA from bovine samples was depicted in the form of scatter diagram (Fig. 25), in which more number of positive cases was scattered between 40 and 60 %, which indicates the sero-prevalence of the PPR in cattle at basal level in most of the cases. In the distribution of the PPRV antibodies in bovines (Fig. 26), 80 samples were having the PI value ranges from 40-60 and 19 strong positive samples had ranges from 60-90. There was no significant difference was observed between the cattle and buffaloes in the percentage positivity for PPR antibodies. High significant difference (P<0.01) between the sheep and goat samples was observed. High percentage of positivity was observed in the sheep, which could be correlated with population of sheep in southern region as reported earlier as well the use of the live attenuated vaccine for PPR control programme in Karnataka state, which leads to reduction in the sample size directly the availability of the un-vaccinated population of sheep and goats. In all earlier studies, antibody sero-prevalence detected in cattle, buffalo and camel from different country confirmed natural transmission of PPR virus under field conditions from sheep and goats to bovines.

Table 2. Sero-prevalence of PPR in India

IVRI, Mukteswar									
Hissar	111	37	20 #	86	16	2#	77.4	43.2	10.0
CADRAD* IVRI	5	28	58	-	-	19	-	-	32.7
Kartholi, Jammu	26	13	-	2	13	-	7.7	100	-
Nammakal, TN	-	2	-	-	-	-	-	-	-
Guwahati,Assam	-	27	11**	-	18	1	-	66.6	9
Ajmer, Rajasthan	22	25	-	10	18	-	45.5	72.0	-
Parbhani,Maharashtra	-	92	-	-	33	-	-	35.9	-
Bareilly UP	92	7	--	59	-	--	64.1	-	-
Palampur, HP	14	16	--	11	10	--	78.5	62.5	-
Mathura, UP	7	38	-	1	26	--	14.2	68.4	-
Agartalla	--	7	--	--	1	--	--	14.3	-
Gujrat	70	--	--	14	--	--	20	--	-
Mannuthy, Kerala	-	30	-	-	5	-	-	16.7	-
Ranchi	-	17	-	-	13	-	-	16.5	-
Kolkata	-	21	-	-	3	-	-	14.3	-
Karnataka	-	31	-	-	15	-	-	48.4	-
Total	**347**	**391**	**89**	**183**	**171**	**22**	**52.7**	**43.7**	**24.7**

O-Ovine-Sheep; C-Caprine-Goat; B-Bovine-cattle. *83 samples were received from CADRAD, that did not have a suitable code, of these, 50 were positive and 23 were negative. .# Donkey sample; ** Camel samples

Table 3. Clinical prevalence of PPR in India

Place/District/State	Samples tested			Samples Positive			Percent (%) positivity		
	O	C	B/o	O	C	B/o	O	C	B/o
IVRI, Mukteswar									
Hissar	56	18	-	26	11	-	46.4	61.1	-
CADRAD*	8	79	-	7	23	-	87.5	29.1	-
Pune	-	10	-	-	2	-	-	20	-
Kartholi	11	12	-	-	-	-	-	-	-
Tamilnadu	-	3	-	-	-	-	-	-	-
Maharashtra	-	65	-	-	14	-	-	21.5	-
Palampur	-	21	-	-	2	-	-	9.5	-
Mathura	-	10	-	-	1	-	-	10.0	-
Agartalla	-	06	-	-	1	-	-	16.66	-
Guwahati	-	03	-	-	0	-	-	-	-
Gujrat	-	-	16#	-	-	1	-		6.3
Jammu	2	-	-	-	-	-	-	-	-
Avikanagar, Rajasthan	5	-	-	-	-	-	-	-	-
Total	82	227	16	33	52	1	40.24	22.9	6.3

O-Ovine-Sheep; C-Caprine-Goat; B/o-Bovine-cattle/others. *samples were received from CADRAD that did not have a suitable code. # Leopard samples-3 ; Camel samples-13

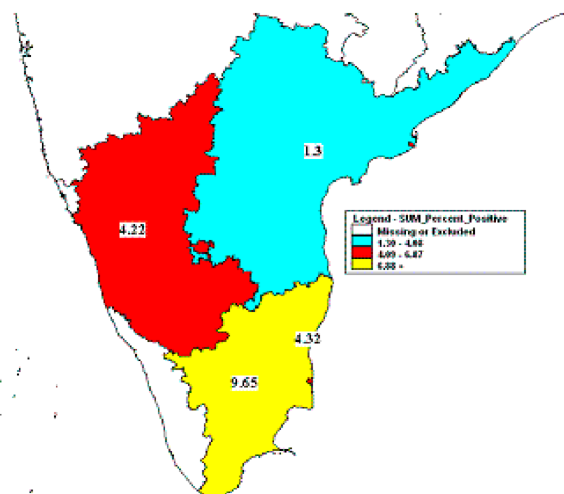


Fig. 24. Sero-prevalence of PPR in Bovine in Southern Peninsular India (Andhra Pradesh, Karnataka, Puduchery and Tamilnadu)

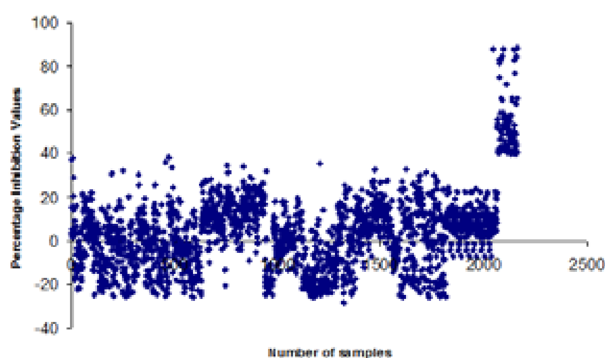


Fig. 25. PPRV antibodies in the cattle serum samples

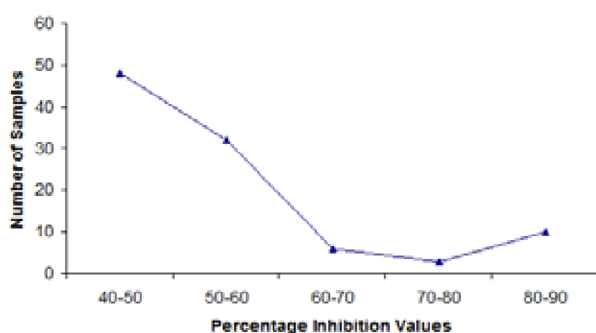


Fig. 26. Distribution of PPRV antibodies in cattle population

The variations in sero-prevalence could also be due to differences in sample size, age, prevailing managemental practices, humidity and season. Serological evidence for the transmission of PPR virus from sheep and goats to cattle and highlights the need to include PPR serology in the sero-monitoring programme to give a better indication of national herd immunity. Experimental transmission of PPR virus from sheep and goats to susceptible cattle has been demonstrated. Cattle infected experimentally with PPRV showed no clinical signs but developed a humoral antibody response to PPRV. Similarly, our earlier study on sub-clinical PPR infection in experimental cattle showed that PPRV antibody could be detected over a period of one year (unpublished data). Also, cattle infected by contact with PPR infected sheep and goats developed humoral antibody against PPRV and were protected against challenge with virulent rinderpest virus. The results presented in this study showed serological evidence for the transmission of PPRV from sheep and goats to cattle under natural conditions.

In conclusion, the per cent positivity of the antibodies in cattle indicates sub-clinical status of the disease which is attributed to variations in the husbandry practices within different geographical regions, the agro-climatic conditions, topography of different states, the socio-economic status of individual farmers and the migration of livestock in India. The presence of PPRV antibodies in situations, where the sub-clinical infection was suspected in cattle and buffaloes indicates bovines are exposed to PPR infection in naturally either directly or indirectly. The transmission of PPR from small ruminants to cattle may be dependent on the type of animal husbandry and possibly the strain of PPRV circulation in geographical areas. Further, systematic studies on sero-epidemiological aspect are to be planned to examine these factors in precipitation of disease in cattle and buffaloes including other ruminants.

Serum samples from different states of the country through coordinating units of PD_ADMAS, submitted to the serum bank of the PD_ADMAS, Bengaluru were also subjected to screening of PPRV antibodies by using the PPR-cELISA kit. The over all details of the results are presented in the Table 4 and percent positivity in graph (Fig. 27). Further, collection of serum samples of different livestock species from other states of southern India and screening of the collected cattle serum samples, submitted samples to the institute against PPRV antibody by using PPR cELISA are in progress.

Table 4. The results of serum samples screened for PPRV antibodies

State	Cattle	Positive	Buffalo	Positive	Sheep	Positive	Goat	Positive	Total	Positive
AndhraPradesh	280	5	40	2	42	27	45	20	407	54
Karnataka	1279	55	1001	44	416	108	92	9	2788	216
Manipur	200	-	-	-	23	-	167	-	390	-
Puduchery	440	19			-	-	-	-	440	19
Tamilnadu	259	25	-	-	-	-	18	2	277	27

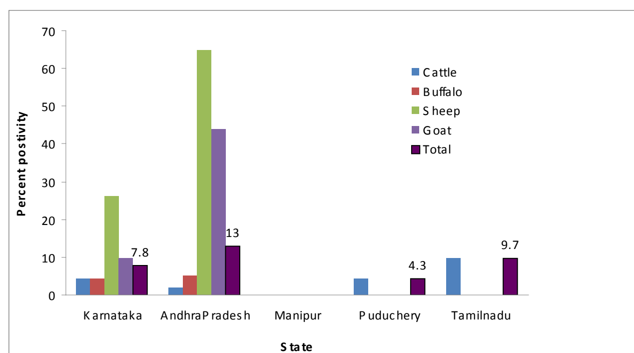


Fig. 27. Seroprevalence of PPR in India

Project: 5

Epidemiology of Bovine Herpes Virus-1 infection

S. S. Patil and Divakar Hemadri

Among alpha herpesviruses infecting ruminants, the bovine herpes virus 1 (BoHV-1) is a important pathogen of cattle, which has worldwide distribution. Based on antigenic and genomic characteristics, BoHV-1 has been further subdivided in two distinct yet closely related subtypes: 1 (BoHV-1.1) and 2 (BoHV-1.2). It has also been proposed that such subtypes may be associated to distinct clinical manifestations of disease in cattle: subtype 1 causes respiratory tract infections known as infectious bovine rhinotracheitis (IBR), while, the subtype 2 has been associated to genital diseases known as “infectious pustular vulvovaginitis” (IPV) or balanoposthitis (IPB) as well as other forms of reproductive failure.

Since, different types and subtypes of bovine herpesvirus 1 have been shown to be associated to different clinical conditions of cattle differentiation of type/subtype becomes essential for understanding the pathogenesis and epidemiology of BoHV infections.

In this study, Eight IBR/IPV suspected clinical samples (Nasal, conjunctival, vaginal and prepucial swabs) collected from cattle/buffaloes (details are given in Table 5) along with three other isolates (ADMAS-1, ADMAS/258-08 and Ooty) from our repository were processed for viral DNA extraction, PCR and partial nucleotide sequencing of the gB gene. Before nucleotide sequencing, the identity of these PCR products (443 bp) was confirmed by digestion with restriction enzyme, *Dde* I, which yielded two fragments of size 346bp and 97bp. The aim of the present study was to generate baseline information about Indian BoHV-1 subtypes and also to increase our understanding about the genetic relatedness of Indian BoHV-1 with other bovine alphaherpesviruses (BoHV-1.1, 1.2 and 5) based on the analysis of the gB gene region.

Aligned nt sequences revealed high degrees of homology in all alphaherpesviruses compared in the study. The nucleotide sequence identity among the Indian strains varied from 99-99.5%, so was their identity with Cooper strain. The nucleotide sequence homology among the strains/isolates of subtype 1.1 was 97.8 to 100 %. In the aligned region, the Indian isolate, Ooty differed from the reference Cooper strain by one nucleotide (position 56483, A T) while PD_ADMAS-1 in addition to the above position (A C) also differed at position, 56485 (C T). Similar to the nucleotide sequence aligned, amino acid sequences revealed high degree of identity in all the BoHV-1 isolates used in the study.

The phylogenetic tree (Fig. 28) inferred by the neighbor-joining method allowed the grouping of viruses according to their respective types or subtypes as previously reported. It is evident from the figure that the strains of BoHV-1 have grouped in two different nodes; one related to BoHV-1.1 and another to BoHV-1.2. All the Indian strains used in the study belong

Table 5. Details of clinical samples/isolates used in the study

Sl. No	Acc No	Source	State
1	ADMAS/258-08	Div. Virology, Mukteswar , (Isolated from Conjunctival swab)	UP
2	ADMAS/685-10	CB-Bull semen-	Orissa
3	ADMAS/688-10	CB-cow-vaginal swab	Orissa
4	ADMAS/723-10	Buff heifer-Nasal swab	Karnataka
5	ADMAS/742-10	IAHVB, Kolkota	West Bengal
6	ADMAS/743-10	Indigenous bull-semen	Karnataka
7	ADMAS/744-10	Indigenous bull-semen	Karnataka
8	ADMAS/745-10	Indigenous bull-semen	Karnataka
9	ADMAS/746-10	Indigenous bull-semen	Karnataka
10	Ooty	Bull semen	Tamilnadu
11	ADMAS-1	Nasal swab	Karnataka

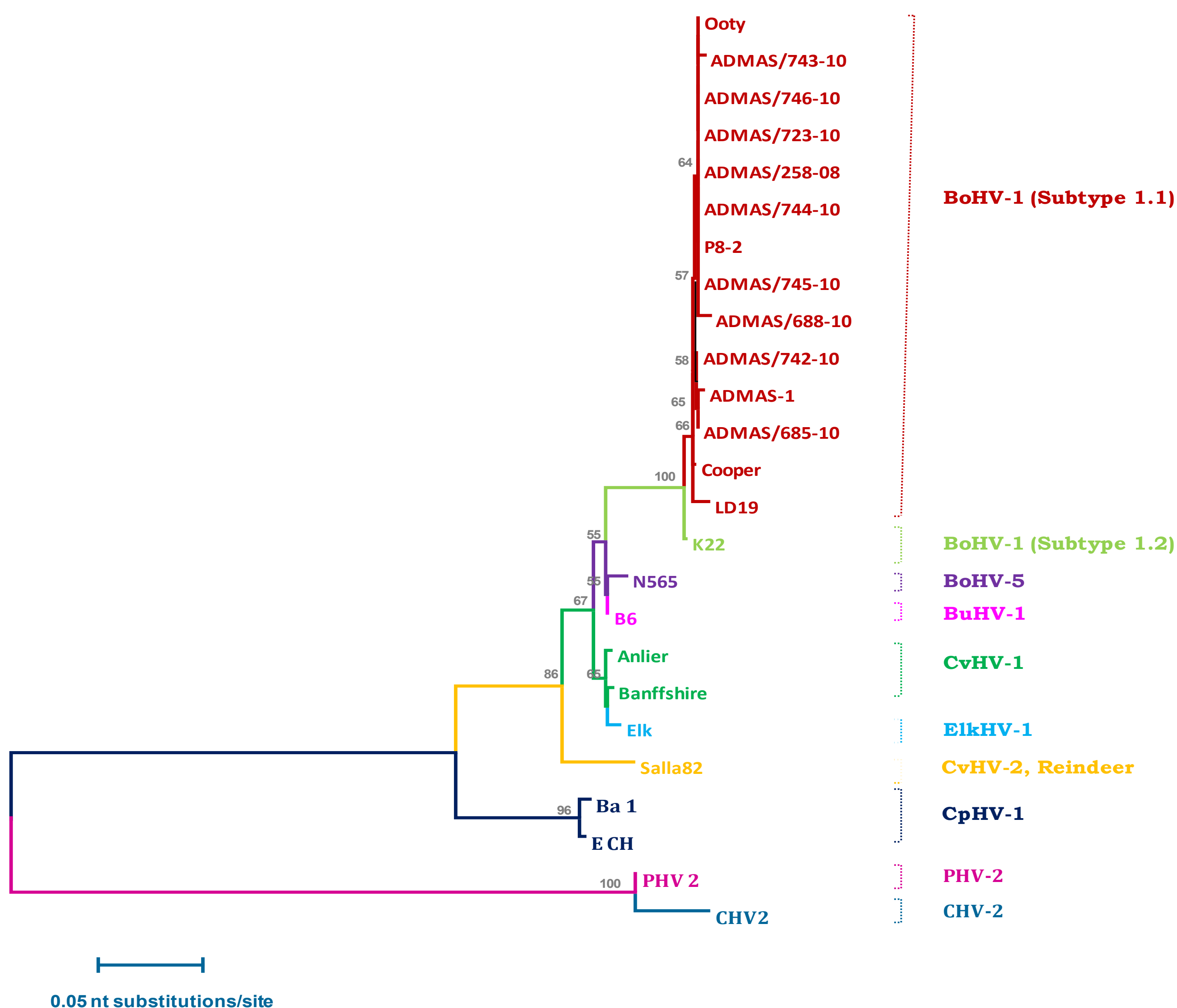


Fig. 28. Neighbour Joining tree showing grouping of Indian BoHV-1 isolates with other Herpes Viruses
The number at the nodes indicate bootstrap (1000 replicates) values. Only values above 50% are shown.

to subtype 1.1 irrespective of animal species, clinical manifestations. The major prevalent group in India taken together, the phylogenetic analysis indicates that BoHV1.1 is the predominant subtype prevalent in India.

Cumulative Seroprevalence of BoHV-1

During the year 2010-11 a total of 1453 serum samples were screened for the presence of anti-BoHV-1 antibodies using indirect ELISA (PD_ADMAS Kit). The overall prevalence of IBR was found to be 36 % (Table 6, Fig. 29) cumulative study (1995-2010). 57009 serum samples from different parts of the country were tested by AB-ELISA during these years and 20749 samples were found positive. The variation in the overall prevalence of IBR may be attributed to the sample size.

Table 6. Year-wise cumulative report on seroprevalence of IBR

Sl. No.	Year	No. Tested	No. positive	Apparent % positive
1.	1996-97	3521	1096	31
2.	1997-98	1442	599	42
3.	1998-99	1675	767	46
4.	1999-01	6883	2776	40
5.	2001-02	3373	785	23
6.	2002-03	7933	3271	41
7.	2003-04	1300	668	51
8.	2004-06	9564	3507	37
9.	2006-07	2820	1197	42
10.	2007-08	4270	1242	29
11.	2008-09	4821	1423	30
12	2009-10	4496	1494	34
13	2010-11	1483	621	42
	TOTAL	57009	20749	36

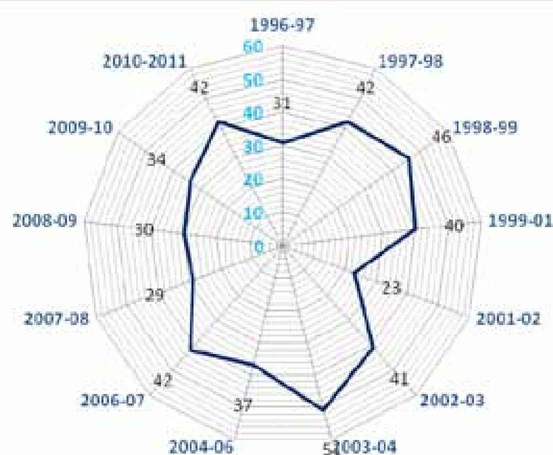


Fig. 29. Seroprevalence of IBR in Bovines during 1996-2011

Project: 6

Seroepidemiology of Classical Swine Fever

S. S. Patil and Divakar Hemadri

India has 13.5 millions of domestic pigs and Southern India, which has 12,80,000 pigs (Livestock Census 2003) is next only to the North Eastern states in numbers. Barring handful of organized farms, the pigs are either reared in backyards (more particularly in North Eastern States) or as small herds by socially and economically backward people of society in other parts of the country. Full potential of Indian pig industry can be realized only if both the production and health aspects of pigs are properly addressed. In this context, a study was undertaken at PD_ADMAS with respect to the most dreadful disease of pigs viz., classical swine fever (CSF) wherein sero epidemiology of CSF virus infection in pigs was studied.

A total of 1257 serum samples collected from four (4) states viz., Andhra Pradesh, Karnataka, Kerala & Maharashtra, were subjected to blocking ELISA using commercial kit (IDEXX, Switzerland). It was evident that a total of 237 serum samples were positive for CSF antibodies. An overall apparent percentage positive was 19. Andhra Pradesh recorded a highest percent of prevalence of CSF antibodies, followed by Kerala (22%), Maharashtra (14%) and Karnataka (12%). (Table 7 and Fig. 30)

Table 7. Seroprevalence of CSFV during 2008-2011-statewise

Sl. No.	States	No. Tested	No. positive	Apparent % positive
1	Andhra Pradesh	498	126	25
2	Karnataka	510	63	12
3	Kerala	166	36	22
4	Maharashtra	83	12	14
	TOTAL	1257	237	19

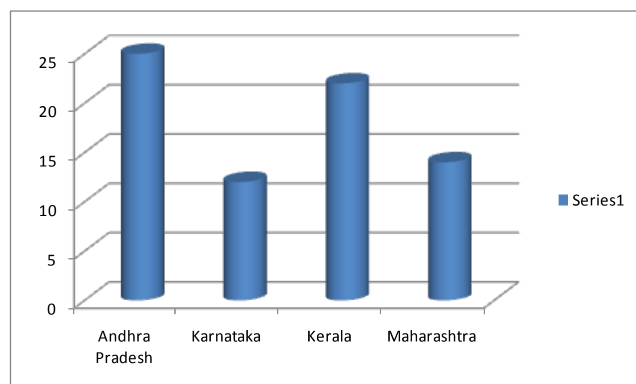


Fig. 30. Statewise sero-prevalence of CSFV in pigs during 2008-2011

Project: 7

Bluetongue disease epidemiology: Spatio-temporal distribution of Culicoides spp and role of cattle & wildlife in bluetongue disease

Mudassar Chanda, Divakar Hemadri and M. R. Gajendragad

Culicoides spp were collected using light trap with UV light and kept from dusk to dawn in the selected farms. Culicoides spp were collected from one cattle farm, one sheep farm and one mixed farm. In one farm the collection was done twice with a gap of one month. GPS readings were taken at each farm and meteorological parameters were extracted. The details are given in Table 8.

There is difference in the number of culicoides species collected in the SLBTC farm. The average temperature recorded at 3 hours interval during first collection was 19.72°C and 25.64 °C during the second collection. The other factor which varied between the two collections was wind speed on the day of collection. The wind speed was more in the second

Table 8. Culicoides species collection from different farms

S. No.	Place of collection	Total number of Culicoides Insects	Other insects	Total
1.	State livestock breeding farm Hessarghatta, Bengaluru (Cattle farm) Ist collection (6/01/2010)	920	1170	2090
2.	State livestock breeding farm Hessarghatta, Bengaluru (Cattle farm) 2nd collection(11/02/2010)	118	1339	1457
3.	Sheep farm Chintamani (20/01/2010)	10800	500	11300
4.	Mixed Farm (Cattle, Buffalo and Goat) 20/02/2010	530	3370	3900

collection which makes difficult for the culicoides to fly and thus there was reduction in the collection. The SLBTC farm comprised of only Cattle and there was no other species of animals. The second farm at chintamani comprised only of Sheep and temperature (20.5^{0}C) and wind speed (7.6 m/s) also favoured the huge number of culicoides captured (10,800). Three collections from the farm in chintamani is still under sorting.

Project: 8

Study on epidemiology and bacterial etiology of infectious abortions in livestock with special reference to brucellosis

Rajeswari Shome and *B.R. Shome*

Brucellosis is reproductive disease that causes abortions and infertility in animals. It is an important zoonotic disease of man and causes chronic debilitating disease and infertility. The objective of the project were

i. Isolation and characterization of bacterial agents, viz, *Brucella spp.* from animals with history of abortion and infertility.

ii. Standardization of indirect-ELISA for sero-screening of brucellosis in sheep and goats.

iii. Technique/s (latex based agglutination test) for diagnosis of brucellosis in livestock.

iv. Direct detection of *Brucella* in milk, infected aborted material, semen, blood and serum by PCR.

v. Nucleotide sequencing of PCR amplified genes (Partial) of *Brucella* for molecular epidemiological studies.

1. Indirect ELISA for diagnosis of brucellosis in sheep and goats

Brucella detection assays for goats and sheep are nearly the same as those for cattle because of the considerable genetic similarity between smooth strains of *B. melitensis* and *B. abortus*.

An indirect ELISA has been standardized using smooth lipopolysaccharaide (sLPS) antigen from *Brucella abortus* S99 and sheep hyperimmune serum (HIS) against sLPS. The dilutions of serum, antigen, and conjugate in iELISA were 1: 100, 1:200, and 1: 10,000 respectively were found optimum for assay. Maxisorp microtitre plates, 2% bovine gelatin as blocking agent and antigen coating for overnight at 4^0C were preferred for the assay. Rabbit anti-goat IgG was preferred over rabbit anti-sheep IgG HRP conjugate. The analytical sensitivity of HIS in iELISA was found upto 1:4 09600 dilution of antibody. The OIE and National *B. abortus* reference (IVRI, Izatnagar) positive and negative serum samples showed the OD values within the defined upper and lower control limits. The positive diagnostic cut-off was set at >54 and negative cut-off which is the average PP values of negative sera was calculated as < 22.

The diagnostic sensitivity (Dse) and diagnostic specificity (Dsp) were found to be 95.66% and 96.33%, respectively when compared with imported VMRD kit (Table 9). The inter and intra–institutional validation score of 90 to 95%, respectively were achieved with the coded samples and the reagents supplied in the kit.

Table 9. Relative DSe and DSp of the standardized iELISA

iELISA / VMRD	Positive	Negative	Total
Positive	287	13	300
Negative	11	289	300
Total	278	322	600

DSe (True positive/ Total positive) = 287/300 = 95.66%
DSp (True negative/ Total negative) = 289/300 = 96.33%

The developed assay was applied to screen anti-brucella antibodies in goats (n=2362) and sheep (n=1702) samples of different states of India. The prevalence of brucellosis was found to be 8.85% and 6.23% in goats and sheep, respectively (Table 10). When the samples were compared state wise by iELISA, highest seroprevalence is recorded in goats of Madhya Pradesh and Bihar and in sheep population of

Table 10. Seroprevalence of brucellosis in sheep and goats by iELISA

Sl. No.	State	No. goat samples	No. goat positives by iELISA	No. sheep samples	No. sheep positives by iELISA	Total No. of sheep and goat samples	Total No .of sheep and goat positives
1	Bihar	120	36 (30.00)	0	0 (0.00)	120	36 (30.00)
2	Gujarat	924	49 (5.30)	539	24 (4.45)	1463	73 (4.99)
3	Jammu and Kashmir	20	0 (0.00)	0	0 (0.00)	20	0 (0.00)
4	Karnataka	99	10 (10.10)	733	59 (8.05)	832	69 (8.29)
5	Madhya pradesh	162	64 (39.51)	0	0 (0.00)	162	64 (39.51)
6	Maharastra	207	14 (6.76)	71	2 (2.82)	278	16 (5.75)
7	Manipur	80	6 (7.50)	0	0 (0.00)	80	6 (7.50)
8	Rajasthan	229	16 (6.98)	163	12 (7.36)	392	28 (7.14)
9	Tamil Nadu	403	13 (3.23)	194	9 (4.64)	597	22 (3.68)
10	Uttar Pradesh	118	1 (0.84)	2	0 (0.00)	120	1 (0.84)
	Total	**2362**	**209 (8.85)**	**1702**	**106** (6.23)	**4064**	**315 (7.75)**
	Mean ± SE	-	**8.85 ± 4.14**	-	**6.23 ± 1.02**	-	**7.75 ± 4.14**
	CI at 95%	-	**0.74 - 16.96**	-	**4.24 – 7.22**	-	**0.36 – 16.96**

Fig. 31. Seroprevaleence of brucellosis in Sheep by iELISA

Fig. 32. Seroprevalence of brucellosis in Goats by iELISA

Karnataka and Rajasthan, respectively (Fig. 31 and 32). Brucellosis was not recorded in sheep of Uttar Pradesh and in goats of Jammu and Kashmir states.

Comparative evaluation of iELISA with standard culture method and PCR using sero-positive and seronegative sheep and goat samples was carried out. Genus-specific 223bp product could be amplified from 26 vaginal, two blood and six serum samples of sero-positive sheep. Similarly, PCR positives were found in 14, 6 and 1, vaginal, serum and blood samples, respectively from sero-positive goats (Fig. 33). *Brucella* organisms were isolated from thirteen sero-positive sheep samples only. The amplicons of size 223bp and 731bp for genus and species-specific PCR respectively confirmed the 13 cultures as *B. melitensis*. This finding is consistent with the *Brucella* species identified by bacteriological examination with that of species-specific PCR.

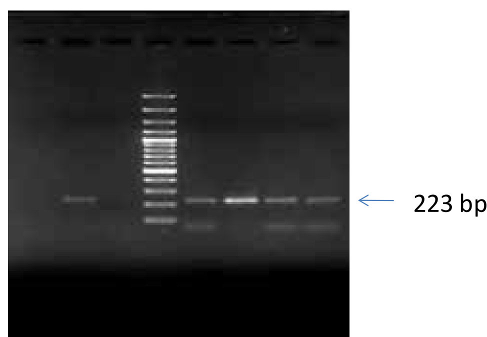


Fig. 33. 1-NTC, 2-Positve sample, 3-Negative sample, M-Marker, 4-7 Clinical samples

The preliminary study revealed the prevalence of brucellosis to be 7.75% in small ruminants. The iELISA assay will be of help to screen large number of small ruminants and which in turn help to implement comprehensive control policy of brucellosis not only in cattle but also for small ruminants.

In the cross reactivity studies, *E. coli* (O157: H7), seventeen *Salmonella* and five *Y. entericolitica* serotypes-specific serum samples found negative by iELISA indicating no cross reactivity problems in the standardized assay (Fig. 34).

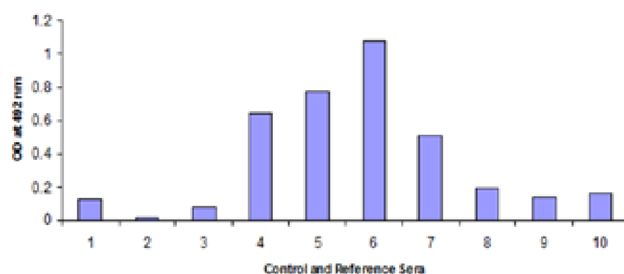


Fig. 34. Reactivity of different control and reference sera with sLPS antigen 1: *Y. enterocolitica*; 2: *E. coli* O157; 3: *Salmonella*; 4: OIE positive reference sera; 5: Indian Veterinary Research Institute (IVRI) positive national reference sera; 6: anti sLPS strong positive sera; 7: anti sLPS moderate positive sera; 8: i ELISA negative control sera; 9: OIE negative reference sera; 10: IVRI negative reference sera

Provisional patent No. 01592/CHE/2008 has been obtained.

The kit is available with 250 and 450 test format and will be provided on request for diagnosis of brucellosis in sheep and goat.

2. Indirect ELISA for diagnosis of brucellosis in swine

Occurrence of swine brucellosis is widespread throughout pig-rearing world, but prevalence is generally low. Brucellosis in swine is routinely diagnosed by rose bengal plate test (RBPT) and standard tube agglutination test (STAT).

A polyclonal antibody-based indirect ELISA (iELISA) for the detection of anti-brucella antibodies in swine was standardized using sLPS antigen from *Brucella abortus*. The average OD values of 1.08±0.13, 0.506± 0.09 and 0.19±0.06 were calculated as the inter-assay difference for strong, moderate, and negative controls, respectively. The PP values of above 45 and less than 22 were taken respectively as positive and negative cut-off values. The diagnostic sensitivity and diagnostic specificity were found to be 95.70% and 74.71%, respectively.

For the evaluation and confirmation of sero-positivity 300 pig blood and sera samples were tested. In PCR screening, genus-specific 223 bp products could be amplified from vaginal / blood/serum samples of 105 pigs (Fig. 35). In isolation, 5 isolates identified as *B. suis* and confirmed by Bruce ladder species specific PCR. Biotyping by AMOS PCR, three out of five isolates were grouped as *B. suis* biovar-1 and rest two isolates to any one of the biovar 2 to 5.

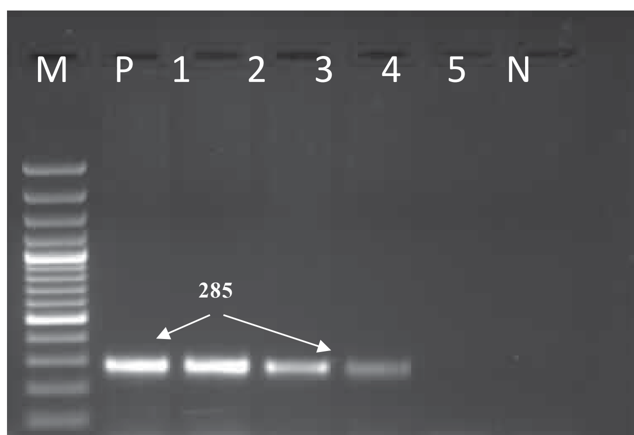


Fig. 35. M Marker; N Negative sample P ;Positve control; 1-5 clinical samples

Among 1759 sera samples from seven states tested using the standardized assay, 270 (15.35%), 84 (4.78.63%) and 389 (22.11%) were positive for *Brucella* antibodies by RBPT, STAT and indirect ELISA respectively. Significant STAT titres of 1:160 (320 IU/ml) in 84 (4.78%) and non-significant titres in 186 (10.57%) were observed among RBPT positive sera samples. Out of three serological tests, RBPT, STAT and ELISA, the iELISA detected higher positives than the other two tests 389 (22.11 %) and significant (P<0.05) difference between the three diagnostic tests was observed.

The farm-wise sero-prevalence when compared, high percent prevalence of disease was recorded in farm-1 of Andhra Pradesh (53%); followed by farms -1 & 2 of Karnataka (59%; 38.46%), Kerala (33%), and Punjab (29.67%), farm-3 of Karnataka. (23.52%), farm-7 of Karnataka (8.06%), farm-2 of Andhra Pradesh (5.88%) and Gujarat (2.48%) states. Anti *Brucella* antibodies were not detected in sera samples of Manipur, Meghalaya and farm-8 of Karnataka (Table 11 and Fig. 36). There is no significant difference in the percentage positive of brucellosis between the seven states at 95% confidence interval and overall prevalence of brucellosis in swine population is shown in the map (Fig. 37).

Table 11. Relative diagnostic sensitivity (DSe) and specificity (DSp) of the standardized indirect ELISA

Indirect ELISA / RBPT	Positive	Negative	Total
Positive	67	38	105
Negative	3	109	112
Total	70	147	217

DSe (True positive/ Total positive) = 67/70 = 95.70%
DSp (True negative/ Total negative) = 109/147 = 74.71%

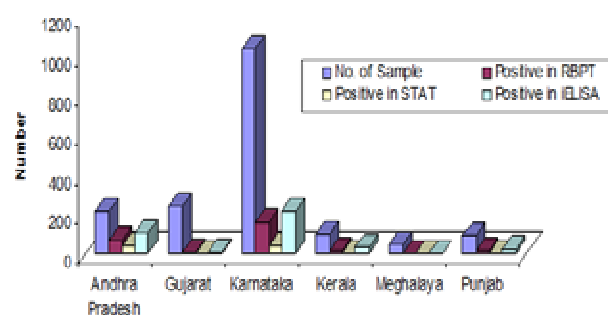


Fig. 36. Seroprevaleence of brucellosis in pigs by different serological tests

In our study, a pig farmer and two male handlers from a pig farm were tested brucellosis positive by RBPT, STAT, PCR and human IgG ELISA. This indicates potential zoonotic threat of the disease to workers associated with swine husbandry and pork industry. This study clearly indicated the difference in the prevalence of the disease between the farms within

the state and high prevalence within the farms may be attributed to both horizontal and vertical transmission of the disease.

Fig. 37. Seroprevalence of brucellosis in Pigs by iELISA

The prevalence of swine brucellosis was found higher with RBPT- 15.35% and ELISA-22.11% than that of small ruminants (7.75%). The history of abortion storms in the pig herd, high seropositivity recorded in the study and isolation studies clearly states that swine brucellosis requires attention. The assay is very useful for sero-screening of swine brucellosis in random samples from swine population (13.51 million) in brucellosis control network programme in the country.

3. Brucella culture repository

a) Isolation

The most compelling direct evidence of brucella infection is obtained by isolation of the causative agent responsible for brucellosis in livestock and humans. *Brucella* isolation protocol using selective broth and selective agar media along with antibiotics was used for preliminary isolation of *Brucella* organisms from clinical samples and vaginal swabs.

From cattle, 27 *B. abortus* out of 50 clinical samples; in small ruminants, 13*B. melitensis* from 33 vaginal samples; one out of 17 human blood samples and in pigs 6 of 26 yielded Brucella organisms. Out of 47 field isolates 28, 14 and 5 belong to *B. abortus, B. melitensis* and *B. suis*, respectively (Table 12). In AMOS PCR, amplified product was observed in all the 28 *B. abortus* isolates and three out of five *B. suis* isolates. It was concluded that all the 28 *B.abortus* isolates belong to any one of the biovar 1, 2 or 4 and three *B. suis* isolates belong to biovar 1 .The remaining two *B. suis* isolates belong to any one of the other four biovars except biovar1. The isolation protocols and typing of isolates and biovars by multiplex PCR are standardized

b) Molecular epidemiology of Brucella organism

PCR-RFLP was carried out using reference *B. abortus* strains (S45/20, S19 and S99), *B.* suis 1330 and *B. melitensis* 16 M along with 10 field isolates. *B.abortus* and *B. melitensis* isolates and reference strains produced similar fragment pattern after digestion of OMP-2b genome amplicon with *Pst1, Dde1* and *Cla1*. However, OMP-2a amplicon of *Brucella* isolates and reference strains showed a distinct pattern after digestion with *Pst1* and *Dde1* that could be able to differentiate some of the *Brucella* species. PCR-RFLP of OMP-2a gene is most important to distinguish different spices of Brucella except *B. canis* and *B. suis* bv3. Hence the target sequence for OMP-2a and -2b for 5 reference and 10 field isolates used in the study amplified the desired 1100bp and 1127 products respectively.

Phylogenetic tree of Brucella isolates-OMP-2b gene based sequences were analysed and studied. Genbank accession numbers were obtained for

Table 12. Species identification of *Brucella* isolates by multiplex Bruce ladder and AMOS PCR

Isolate No.	No. of isolates	Host	Species identified by Multiplex Bruce ladder PCR	Biovar differentiation by AMOS PCR
ADMAS BRU 1, 2, 4-21, 41-43, 45	24	Cattle	*B. abortus*	Biovar 1,2 or 4
ADMAS BRU 38-40	3	Buffalo	*B. abortus*	Biovar 1,2 or 4
ADMAS BRU 22-34	13	Sheep	*B. melitensis*	Any Biovar1-3
ADMAS BRU 35, 36, 44	3	Pig	*B. suis*	Biovar1
ADMAS BRU 46,47	2	Pig	*B. suis*	Except Biovar1
ADMAS BRU 3	1	Pig	*B. abortus*	Biovar 1,2 or 4
ADMAS BRU 37	1	Human	*B. melitensis*	Any Biovar1-3

29 isolations obtained from cattle, sheep, goat, pig and humans. *B. abortus* species specific sequences = 2, *B. melitensis* species specific sequences = 4; *B. suis* species specific sequences =2, OMP (2 a) for *B. melitensis* sequences = 6 ;OMP (2 a) *B. abortus* sequences =23

4. Zoonotic studies of brucellosis

a) Diagnosis of human brucellosis by standardized IgM and IgG ELISA

A total of 499 sera samples comprising of 179- risk group, 123 – blood donors, 197- PUO cases were collected and screened. Out of 179 risk groupsera, 66, 99 and 54 samples were collected from people belonging to veterinary profession, animal handlers and paravets, respectively. Among the 179 sera samples from risk groups (Veterinarians) screened for brucellosis, 10(5.58%) and 4 (2.23%) were positive for *Brucella* antibodies by RBPT and STAT respectively. In IgM and IgG ELISA, 4 (2.23%) and 31 (17.3%) were detected positive respectively. High sero prevalence in the risk group is attributed to constant exposure to infection due to contamination of hands while handling animals and through aerosol.

In case of blood donors, out of 123 samples tested, 2 (1.62%) and 6 (4.87%) were positive by RBPT and IgG ELISA respectively. This infection in the donors might be due to the exposure of the donors unintentionally to the animals or due to the consumption of raw milk, or may be due to the cross reacting antibodies such as *Vibrio* or *Yersinia.*

In case of Pyrexia of unknown origin (PUO) patients sera samples, a total 197 samples were analyzed, out of which, 34(17.25%), 2(1.01%), 1(0.5%) and 23 (11.26%) were found positive by the RBPT, STAT, IgM ELISA and IgG ELISA respectively (Table 13). In the present study, the higher sero prevalence of anti- *Brucella* antibody was detected ranging from 7.61% (RBPT) to 11.67% (IgG ELISA)in POU cases. This is attributed to the collection of samples from diagnostic laboratories located in Bengaluru rural areas where intensive dairy is practiced or exposure might be due to animal handling (farmers) or consumption of unpasteurized milk.

b) Diagnosis of human brucellosis by PCR

For comparative evaluation of PCR vis-a vis serological tests, another set of 219 blood and serum samples were collected in the. Among 219, 98% and 2%; 58.6% and 41.4%; 77.8% and 22.20% were males and females from veterinary profession, animal handlers and suspected cases respectively (Table 14, 15 and 16). In different diagnostic tests that were

Table 13. Diagnosis of human brucellosis by different serological tests

Sample	Total No. of samples	RBPT +ve	STAT +ve		ELISA +ve	
			phenol	2ME	IgM	IgG
Risk group	179	10 (5.58%)	4 (2.23%)	1 (0.5%)	4 (2.23%)	31 (17.3%)
Blood donors	123	2(1.62%)	-		-	6(4.87%)
POU	197	15 (7.61%)	2 (1.01%)	-	1 (0.5%)	23 (11.67%)
Total	499	27 (5.41%)	6 (1.2%)	1 (0.5%)	5 (1.0%)	60 (12.02%)

Table 14. Sex-wise seroprevalence of brucellosis in human by different serological tests

Sex	No. of samples	RBPT	STAT	ELISA	
				Ig M	Ig G
Male	165 (75.34)	27 (16.36)	15 (9.09)	4 (2.42)	47 (28.49)
Female	54 (24.65)	5 (9.26)	3 (5.56)	0 (0)	6 (11.11)
Total (Percent positive Mean ± SE)	219	32 (14.61±0.34)	18 (8.22±0.17)	4 (1.83±0.12)	53 (24.20±0.83)
95% Confidence interval		13.94-15.28	7.88-8.56	7.88-8.56	22.57-25.83

Figures in parenthesis indicate percentage

Table 15. Source wise seroprevalence of brucellosis in human

Source of samples	No. of samples	RBPT	STAT	ELISA	
				Ig M	Ig G
Veterinary doctors & Assistants	66	19 (28.79)	12 (18.18)	2 (3.03)	31 (40.79)
Animal handlers	99	3 (3.03)	2 (2.02)	1 (1.01)	10 (13.16)
Suspected cases	54	10 (18.52)	4 (7.41)	1 (1.85)	12 (15.79)
Total (Percent positive Mean ± SE)	219	32 (14.61±0.88)	18 (8.22±0.56)	4 (1.83±0.07)	53 (24.20±1.03)
95% Confidence interval		12.89-16.33	7.12-9.32	7.12-9.32	22.18-26.22

Figures in parenthesis indicate percentage

Table 16. Prevalence of brucellosis by PCR and isolation in human

Source of samples	Total no. of samples	Genus specific PCR	No. of RBPT positives	Isolation
Veterinary doctors & Assistants	66	7 (10.61)	19	1 (5.26)
Animal handlers	99	2 (2.02)	3	1 (33.34)
Other suspected cases	54	5 (9.26)	10	0 (0)
Total	219	14 (6.39)	32	2 (6.25)

Figures in parenthesis indicate percentage

employed, 53 (24.20%), 32 (14.61%), 14 (6.39%) and 4 (1.83%) were positive in IgG ELISA, RBPT, PCR and IgM ELISA, respectively, in the descending order of positivity.

The high number of positives was detected in male and veterinary professionals. Out of 32 RBPT positive samples, 18 (56.25%) showed significant antibrucella antibody titres by STAT. From the same RBPT positive samples, *B. abortus* and *B. melitensis* were isolated and confirmed by bacteriological, species specific PCR.

Diagnostic anti *Brucella* antibody titres in 18 (56.25%), 223bp amplicon specific for *Brucella* genus in 14 (43.75%) and *Brucella* organisms in 2 (6.25%), were observed (Fig. 38). The present study confirms brucellosis in blood and serum of 14 (6.39%) cases in risk groups. Hence both antibody and antigen detection assays are required to have a foolproof diagnosis to advice for the treatment in human.

c) Diagnosis of human brucellosis by Recombinant bp26 protein based ELISA

Several recombinant outer membrane proteins (OMP) have been tried as diagnostic antigen (s) in ELISA for brucellosis by different research group. In the present study, the ORF (753 bp product) of BP 26 protein was amplified from *Brucella suis* strain 1330, cloned in pET32a vector and expressed in BL21 *E.coli* host cell. The expressed protein was purified by Ni-NTA column and characterized by SDS-PAGE and Western blot analysis.

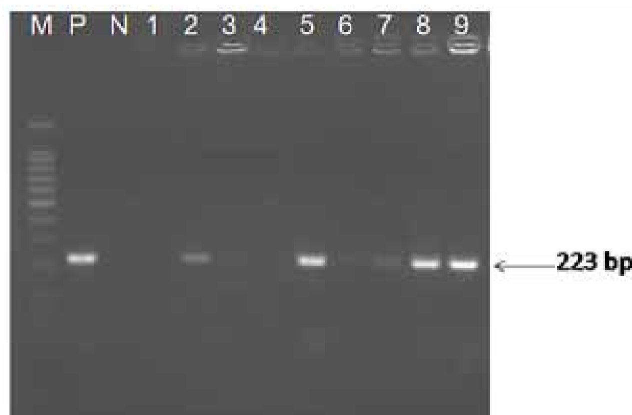


Fig. 38. PCR amplification of 223 bp product of partial bcsp31 gene of Brucella sp. for diagnosis of human brucellosis
Lane M : 100 bp molecular marker
Lane P : 223 bp PCR amplified product from B. abortus S,, strain
Lane N : Non-template control
Lane 2,5,6,7 : 223 bp PCR amplified products from serum/plasma of some humans
Lane 1,3,4 : No amplified product from serum/plasma of humans
Lane 8 and 9: 223 bp PCR amplified products from clinical isolates

The purified recombinant protein (rbp26) antigen was tested in indirect ELISA and was evaluated with serum

samples (n=626, Table 9) collected from risk group individuals (veterinarians) in 2 conjugates (IgM and IgG). Comparative evaluation of the developed assay with RBPT and smooth lipopolysaccharide (sLPS) based ELISA.

Table 17. Results of Samples analyzed

Total Number of Human samples	626
Total No. of Positives (positive in RBPT and all the 4 iELISAs)	53
Total No. of Suspicious (negative in RBPT and positive in any two iELISAs)	20
Total No. of False positives (negative in rbp26 iELISA and positive in RBPT and sLPS iELISAs	15

The high specificity of rbp26 ELISA (by eliminating 15 false positives (2.39%) reiterates that this assay can be used for serodiagnosis of human brucellosis with safety aspects associated with non-handling of *Brucella* species in the laboratory. Further evaluation of bp26 for other livestock and adopting into the Lateral flow assays will help to develop very specific penside tests for brucellosis.

5. Common ELISA for diagnosis of brucellosis in livestock and human

Major problems in sero-diagnosis of brucellosis by ELISA in livestock species is use of multiple conjugates and maintaining the quality control of each test is difficult. To overcome the problem of using different iELISA protocols, a common ELISA for the detection of antibrucella antibodies in both livestock and humans using recombinant protein G which reacts with immuoglobulins of different species like cow, sheep, goat, swine, human, has been standardized.

On comparative evaluation of 400 sera samples (50 each positive and 50 negatives from cattle, sheep and goat, pig and humans), the diagnostic sensitivity of the test varied from 100% in pigs followed by 92% in both human and small ruminants and 88% in cattle. The diagnostic specificity ranged from 100% in humans and cattle and 96% in pigs and small ruminants. The repeatability of the assay has been with paired sera from 138 cattle. Out of 138 sera tested. 28 (20.28%) and 31(22.46%) samples showed antibrucella antibodies in the 1st and 2nd collection samples respectively in protein - G ELISA.

When 138 samples were compared individually, 21 samples were found positives in both 1st and 2nd collections. The other 10 samples showed variations with respect to positivity/ negativity in the test from both the collections. The repeatability of the test results was found to be 92.8%. Similarly, another set of cattle sera samples (n=152) tested by Protein-G based indirect ELISA. Out of 152, 13 (8.55%) and 9 (5.26%) samples were positive in 1st and 2nd collection.

Using the same protocol Horse serum samples (n=24 and n=13) also tested and 1 out of 24 and 4 out of 13 sera samples were positive in both RBPT and iELISA indicating that the test can be used for diagnosis of other animals species other than cattle, pig, small ruminants and man. This iELISA protein –G assay is highly useful in Indian context because multiple species are reared in intensive and integrated farming system in the country and it is easy to screen the disease in single test format.

6. Molecular diagnostics for brucellosis

PCR based diagnoses are widely used to detect brucella genus in samples. In the study, clinical materials viz., serum, whole blood and vaginal swabs were collected from livestock species that are found positive for *Brucella* antibodies by one of serological tests (RBPT, ELISA and, STAT) for detection of *Brucella* DNA.

The DNA extracted from all clinical materials was subjected to PCR for amplification of 223 bp of BCSP-31 genome that is specific for *Brucella*. The results showed that the presence of Brucella DNA in 14.11% of the clinical samples (Table 18). Good clinical specimen for PCR in livestock is a vaginal swab, followed by serum and blood sample. In humans, blood and serum were found suitable clinical samples for PCR. The PCR protocol standardized in the laboratory gave satisfactory results and a quick method of antigen detection and non- hazardous.

Outcome of the project

Detection of Brucella DNA in clinical samples by genus specific PCR was standardised.

The iELISA kit for 250 and 450 test format will be provided on request for diagnosis of brucellosis in sheep and goats.

Table 18. Detection of Brucella DNA by PCR in livestock and human

Sl. No.	Species	Total No. samples tested	PCR-positivity	Percentage
1	Cattle*	347	49	(14.12%)
2	Pig*	217	105	(48.38%)
3	Horse	13 + 24 = 37	4 + 1=5	(3.78%)
4	Cattle (milk)	72	Nil	-
5	Cattle (Semen)	123	Nil	-
6	Human**	219	14	(6.39%)
7	Sheep*	300	34	(11.33%)
8	Goat*	300	21	(7.00%)
Total		1615	228	14.11%

* Vaginal, blood and serum samples; ** serum and blood samples

The ELISA test standardized in the PD_ADMAS for sero-screening of swine brucellosis will be done upon request at the institute only.

The human samples will be screened using the standardized IgG ELISA and IgM ELISA at PD_ADMAS upon request.

Future research includes evaluation and validation of common iELISA based on recombinant protein-G to screen brucellosis in single test format for livestock and humans. Evaluation of bp26 in Lateral flow assays to develop very specific penside tests for brucellosis.

Project: 9

Monitoring of leptospirosis: Identification and characterization of Leptospira isolates from livestock and human

V. Balamurugan, M. Nagalingam, P. Krishnamoorthy and K. Prabhudas

Leptospirosis is a zoonotic infection infecting both man and animals caused by Leptospira. Leptospirosis, being a zoonotic disease of livestock with a large variety of animal species acting as carrier, it is difficult to eliminate and perhaps even control in tropical developing countries like India. Diagnosis is difficult as early symptoms resemble symptom of common infections like flu, typhoid, dengue, malaria, etc. Laboratory diagnosis is based on dark field microscopic (DFM) findings, isolation of the organism in EMJH medium, staining technique, microscopic agglutination test (MAT) and molecular techniques. Early case detection or identification and prompt treatment as well as creating awareness about the disease among the people and public health personnel are the steps that could be taken to reduce the extent of the problem. It is imperative to identify and characterize the leptospira isolates from livestock and human from time to time for monitoring of leptospirosis particularly in livestock in the country. This in turn will provide the facility for typing with in the country, specific detection method for the leptospira antigen detection, selection of panel of antigen to be used in the MAT diagnostic method at different geographical location in livestock species and human and also to facilitate monitoring of leptospirosis in livestock species. This project aimed at providing a clear epidemiological picture of leptospirosis in livestock and the zoonotic potential of leptospirosis in India. The objectives of the project are a) To monitor the leptospirosis- to investigate the prevalence of leptospira species in livestock and human b) To develop the molecular based diagnostic method for the detection of pathogenic leptospira of livestock and human.

During the period under report, 191 stock cultures from different livestock and human hosts [Bovine-93 (cattle-82; Buffalo-11)]; Canine 16; Horse-8; Human-31; Goat-11 Elephant-1; Rodent-25; and water body source-6], which were isolated in EMJH medium and whose morphological characters were assessed by dark field microscope as spirochetes were taken up for analysis (Table 19). DNA was extracted from these cultures by using QIamp DNA mini kit and subjected to three different types of PCR reactions for identification of leptospira organisms, which were already standardized {one Leptospira genus specific PCR and two pathogenic leptospira specific PCR ie., *secY* (translocase) and 16s rRNA genes based PCRs} for identification of leptospira organisms.

Initially, Leptospira genus specific PCR based on the 16s rRNA gene using reported primer set was carried out to differentiate the leptospira from other spirochete organisms namely, *Borrelia* and *Treponema, Leptonema* etc., which amplified 331bp products from the leptospira organism only. Hundred (100) cultures were found to be leptospira. Ninety one (91) non leptospira organisms were also recorded, which may be either Borrelia or Treponema or some other spirochetes, which required further study. Further, pathogenic 16s rRNA based PCR using E1 and E2 primers was

performed which amplified the 571 bp amplicon from all the pathogenic leptospira. Then, *secY* based PCR amplified the 285bp product from pathogenic Leptospira species except L. *kirschneri* sero group and Grippotyphosa. Once, pathogenic leptospira isolates were identified, *rpoB* gene specific PCR was carried out to amplify the partial approximate 600 bp product for species identification and further sero-group specific PCR also employed for identification or classify sero-group within the species. eg. Grippotyphosa group in *L. krischneri* species.

Table 19. Details of the *Leptospira* isolates from different Livestock and Human at various state of India taken for the study.

S. No.	Species		Total	Identified
1	Human		31	10
2	Bovine	Cattle	82	21
		Buffalo	11	4
3	Goat		11	5
4	Horse		8	-
5	Rodent		25	7
6	Elephant		1	1
7	Dog		16	4
8	Water bodies		6	-
	Total		**191**	**52**

For characterization of the *Leptospira* species, the *rpoB* partial gene sequences (≈600bp) were amplified from pathogenic leptospira isolates, cloned and sequenced. The PCR amplicons were purified by using QIAquick gel extraction Kit (Qiagen, Germany) as per manufacturer's protocol and cloned into pGEM-T Easy vector (Promega, USA) following standard procedures. The recombinant plasmid DNA was isolated and confirmed by PCR, restriction endonuclease analysis and sequencing, of which the later was carried out commercially in an automated DNA sequencer (ABI 3100, Perkin Elmer). The partial *rpoB* gene sequences of the pathogenic leptospira were obtained after editing the primer sequences. A total of 52 isolates were identified by the sequence analysis of partial *rpoB* gene Table 20.

Analysis of a segment of *rpoB* may be useful as an initial screening test for the identification of a new isolate of leptospira using a system of similarity cut-off to define species. If the partial *rpoB* similarity of a test isolate is lower than 92%, it should be regarded as a new species. On the other hand, if such a value is more than 97%, the isolate under scrutiny should be regarded as being representative of a known species. In general, isolates belonging to *L. borgpetersenii* serovar Hardjo showed 99.8 to 100 % identity with reported

Table 20. Details of identified pathogenic Leptospira isolates from livestock and human

S. No	Register Number	Culture No.	Species	Identification by *rpoB* based analysis (Leptospira Species/sero group/serovars)
1	34	ADMAS 1063	Human	*L.Krischneri* GripphotypOsa
2	32	ADMAS 1183	Elephant	L.Krischneri Gripphotypsa
3	15	ADMAS 1256	Canine	*L.borgepettersenii* Hardjo
4	17	ADMAS 737	Human	*L.borgepettersenii* Hardjo
5	26	ADMAS 1345	Bovine	*L.borgepettersenii* Hardjo
6	28	ADMAS 1590	Bovine	*L.borgepettersenii* Hardjo
7	31	ADMAS 1215	Goat	*L.Krischneri* Gripphotypsa
8	36	ADMAS G73	Bovine	*L.Krischneri* Gripphotypsa
9	19	ADMAS 1178	Human	*L.Krischneri* Gripphotypsa
10	49	ADMAS 1228	Bovine	*L.borgepettersenii* Hardjo
11	21	ADMAS 1175	Bovine	*L.borgepettersenii* Hardjo
12	94	ADMAS G90	Bovine	*L.interrogans* Australis
13	58	ADMAS 58	Human	*L.interrogans icterohaemorrhagiae*
14	91	ADMAS G202	Bovine	*L.interrogans Bratislava*
15	16	ADMAS 1194	Canine	*L.Krischneri* Gripphotypsa
16	27	ADMAS 1762	Bovine	*L.Krischneri* Gripphotypsa

S. No	Register Number	Culture No.	Species	Identification by *rpoB* based analysis (Leptospira Species/sero group/serovars)
17	92	ADMAS G229	Bovine	*L.inadai sub-group*
18	38	ADMAS G70	Bovine	*L.borgepettersenii* Hardjo
19	146	ADMAS 3394	Goat	*L.interrogans Bratislava*
20	109	ADMAS 3398	Goat	*L.interrogans Bratislava*
21	129	ADMAS 1756	Bovine	*L.inadai sub-group*
22	116	ADMAS 107	R. Rouf	*L.interrogans*
23	114	ADMAS 1190	Human	*L.interrogans*
24	111	ADMAS 966	Human	*L.inadai sub-group*
25	139	ADMAS 1332	Rat	*L.inadai sub-group*
26	141	ADMAS 1147	Human	*L.inadai sub-group*
27	145	ADMAS 1761	Buffalo	*L.inadai sub-group*
28	147	ADMAS 2421	Bovine	*L.interrogans Bratislava*
29	149	ADMAS 1856	Bovine	*L.borgepettersenii* Hardjo
30	153	ADMAS 2475	Cow	*L.interrogans* Australis
31	157	ADMAS 421	Buffalo	*L.inadai sub-group*
32	158	ADMAS 3360	Cow	*L.inadai sub-group*
33	161	ADMAS 3395	Goat	*L.interrogans Bratislava*
34	169	ADMAS 2757	Cow	*L.inadai sub-group*
35	175	ADMAS 3334	Cow	*L.inadai sub-group*
36	176	ADMAS 3397	Goat	*L.inadai sub-group*
37	177	ADMAS 2882	Cow	*L.borgepettersenii* Hardjo
38	182	ADMAS 3647	Rat	*L.borgepettersenii* Hardjo
39	189	ADMAS 843	Rodent	*L.borgepettersenii* Tarassovi
40	193	ADMAS 930	Human	*L.Krischneri* Gripphotyposa
41	197	ADMAS 950	Human	*L.borgepettersenii* Tarassovi
42	205	ADMAS 2480	Cow	*L.interrogans Bratislava*
43	206	ADMAS 2636	Canine	*L.interrogans Bratislava*
44	208	ADMAS 2667	Canine	*L.borgepettersenii* Tarassovi
45	210	ADMAS 2713	Baffalo	*L.borgepettersenii* Tarassovi
46	211	ADMAS 2779	Cow	*L.inadai sub group*
47	212	ADMAS 3233	Human	*L.inadai sub group*
48	213	ADMAS 3377	Cow	*L.inadai sub group*
49	215	ADMAS 21	R. hinton	*L.borgepettersenii* Hardjo
50	217	ADMAS 121	Rodent	*L.borgepettersenii* Hardjo
51	218	ADMAS 840	Wilter Rat	*L.borgepettersenii* Hardjo
52	220	ADMAS 1003	Buffalo	*L.borgepettersenii* Hardjo

sequences of *L. borgpetersenii* serovars Hardjo-bovis and Prajitno. Similarly, isolates belonging to *L. interrogans* species showed 99 to 100 % identity with reported sequences of various serovars of species *L. interrogans*. *L. borgpetersenii* serovars Hardjo-bovis Isolates belonging to *L. inadai species* showed only 75 to 78 % identity with reported sequences of *L. inadai* serovar Lyme; Isolates belonging *L. kirschneri* serovar Grippotyphosa had 98-99 % identity with reported sequences.

On phylogenetic analysis of isolates based on *rpoB* gene sequences revealed that 14 isolates belong to *L. borgpetersenii* serovar Hardjo, 8 isolates belong to *L. kirschneri* serovar Grippotyphosa, 16 isolates belong to *L. interrogans* species with major serovar Australis / Bratislava, Tarassovi and Icterohaemorrahagie and 14 isolates belong to *L. inadai* species subgroup. Further, 8 isolates of *L. kirschneri* serovar Grippotyphosa was confirmed by using sero-group specific PCR technique, which yielded a specific 352 bp products (fig. not shown). Based on the present study, the overall percentage prevalence of leptospira species in livestock and human (Fig. 39) are *L.borgpetersenni, L. interrogans, L. inadai and L. Krischneri* 27, 31, 27, 15, respectively were observed. Similarly, among the bovine population, the percentage prevalence of species was 32, 24, 36, and 8, respectively (Fig. 40). In Karnataka, the prevalence rate of these species was 38, 27, 23 and 12, respectively (Fig. 41). The major circulating pathogenic species of leptospira in livestock and human were *L. borgpetersenii* serovar Hardjo, *L. kirschneri* serovar Grippotyphosa *L. interrogans*, serovars Australis, Bratislava, Tarassovi and Icterohaemorrahagie *and L. inadai* species subgroup (Table 21, 22) were observed, the later species requires further study to determine the exact serovars or new species, as recently more classification of new isolates was made for this species. Further, earlier study from our laboratory, showed that, circulation of *L. inadai* in reservoir host in Bengaluru, India. To establish the possible sero-prevalence of this species in the population, the inclusion of *L. inadai* in the battery of leptospiral antigens used for sero-epidemiological studies is recommended. Long term preservation of these pathogenic leptospira cultures under semi-solid EMJH medium and further characterization of these pathogenic isolates are in progress.

The present preliminary study provides an overall prevalence of leptospirosis in livestock species and the zoonotic potential of leptospirosis in India. This could be useful in selection of panel of antigens to be used in the MAT at different geographical location to the extent possible. However, further systematic random screening of the samples to be carried out in order to study further prevalence rate in particular locality, which depend on the various epidemiological factors pertaining to leptospirosis namely contaminated environments, such as water and soil, dehydration, temperatures , rainy seasons etc.,

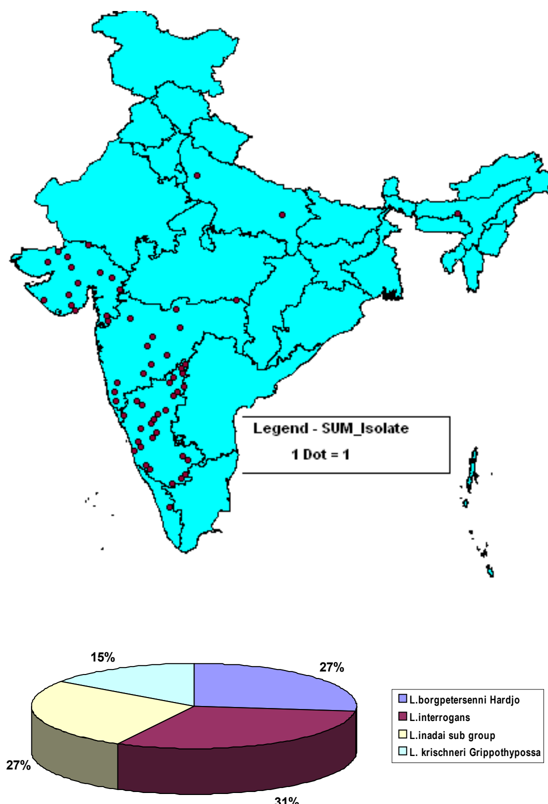


Fig. 39. Prevalence of Leptospirosis in Livestock and Human

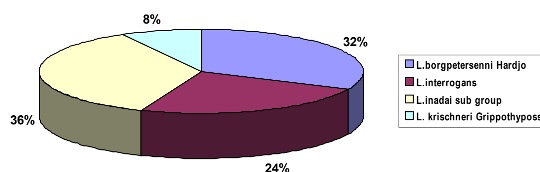


Fig. 40. Prevalence of Leptospirosis in Bovine Population

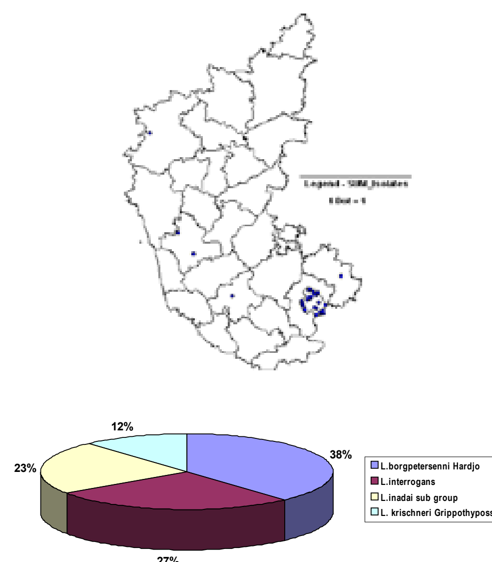


Fig. 41. Prevalence of leptospirosis in livestock and human in Karnataka

Table 21. Prevalence of Leptospira species in India

State	Species	*Leptospira* Species/ Serovars				
		L. borgpettersenni Hardjo	***L. interrogans***	***L.inadai***	***L.krischneri Gripphotyposa***	**Total**
Karnataka	Bovine	5	2	2	-	9
	Human	1	3	3	3	10
	Rodents	4	2	1	-	7
Gujarat	Bovine	1	3	5	-	9
	Canine	-	2	-	-	2
Maharashtra	Bovine	1	1	2	1	5
	Canine	-	-	-	1	1
	Goat	-	3	1	1	5
Uttar Pradesh	Bovine	1	-	-	-	1
	Horse	-	-	-	-	-
Goa	Bovine	-	-	-	1	1
Kerala	Elephant	-	-	-	1	1
Assam	Canine	1	-	-	-	1
Total		**14**	**16**	**14**	**8**	**52**

Project : 10

Production of recombinant immunoglobulin like protein of Leptospira and its evaluation as antigen for diagnosis of bovine leptospirosis

M. Nagalingam, V. Balamurugan, Rajeswari Shome and K. Prabhudas

Leptospirosis is the most widespread zoonosis in the world and has been emerging as an important public health problem in India. The main economic importance of leptospirosis includes direct or indirect costs of abortion, stillbirth, infertility, failure to thrive, loss of milk production, death and associated veterinary costs in domestic and commercial livestock, with potential for malnutrition and impoverishment amongst individuals and communities dependent on animal sources of protein, especially in subsistence economies.

The recent seroprevalence studies indicated that 14.8% seropositivity in cattle in India. The broad spectrum of clinical presentations associated with leptospirosis also hampers case identification. Presence of the complexity in case identification as well as the lack of diagnostic facility hinders us in avoiding losses due to bovine leptospirosis in India. Therefore, early diagnosis must rely on an efficient laboratory test that can be easily implemented in the field without dependence on reference laboratory settings. Unfortunately, there is no such diagnostic facility available commercially throughout the world for bovine leptospirosis.

Microscopic agglutination test (MAT) is the "gold standard" reference test for leptospirosis. Despite its wide-spread usage and international recognition, it has a number of limitations. These include the need to use hazardous live bacteria, expertise to test each serum sample against multiple serovars of this organism and is time consuming. MAT is inadequate for rapid case identification since it can only be performed in a few reference laboratories and requires analysis of paired sera to achieve sufficient sensitivity. Farmers living under poor socio-economical conditions and in rural areas cannot utilize the service rendered by urban based reference laboratories. Rapid diagnostic tests are also not available for diagnosing bovine leptospirosis.

Many standard diagnostic tests except MAT use heat killed non-pathogenic leptospires as antigen which gives high background colour in some tests and less specificity. Recent studies revealed that outer membrane proteins such as immunoglobulin (Ig) like protein is recognized at the early stage of naturally infected animal sera and tests using this recombinant protein antigen has given good specificity and sensitivity because it is only expressed during infection and also only by pathogenic leptospira sp. Rapid diagnostic kit (Latex agglutination test) using these proteins will be having more specificity and sensitivity. This will help in early diagnosis and proper

treatment for bovine leptospirosis which will help in reducing economic losses to farmers and also eliminate carrier animals, thus improving public health status. Hence this project is initiated in August 2010 with the objective of producing recombinant immunoglobulin like protein of leptospira and evaluating it as antigen for diagnosis of bovine leptospirosis.

The leptospiral immunoglobulins like proteins (Lig) are encoded by genes such as *ligA*, *ligB* and *ligC*. Their function is colonizing in host cells by binding to the fibronectin of extracellular matrix of cells and appears to induce strong antibody response. Since intact *ligB* gene is present in many of the pathogenic leptospira compared to *ligA* and *ligC*, *ligB* has been selected as a candidate for producing recombinant protein.

The *ligB* gene is of ~5.7 kb (5670 bp) in length having molecular weight of ~212 kDa. Its signal sequence is 31 amino acids at N terminal. It consists of 12 repeats (D1–D12) of a 90 amino acid motif. The *ligB* conserved N-terminal region, central variable region and C- terminal region consists of 630, 787 and 472 amino acids, respectively. Hence the primers were designed for producing three truncated recombinant proteins from *ligB* gene, so that their sensitivity and specificity can be compared when used in diagnostic tests and the best one can be selected (Table 22)

Cloning of LigB protein coding gene sequences

The DNA was extracted from *Leptospira borgpetersonii* Hardjo isolates (isolated from bovine) by using QIamp DNA mini kit and used for amplifying all the three regions of gene sequences. The conserved (*ligB*con1, *ligB*con2) and variable (*ligB*var) regions of *ligB* gene were amplified by PCR which resulted in specific amplification.

The PCR amplicons were purified by using QIAquick gel extraction Kit (Qiagen, Germany) as per manufacturer's protocol and cloned into either pGEM-T Easy vector or pTZ57R /T vector following standard procedures. The recombinant plasmid DNA was isolated and confirmed by PCR, restriction endonuclease analysis and sequencing, of which the later was carried out commercially in an automated DNA sequencer (ABI 3100, Perkin Elmer). After editing the primer sequences used for PCR amplification the *ligB* gene sequences of the *Leptospira borgpetersonii* Hardjo isolates were determined. The plasmid DNA containing the target gene sequences in the back bone of the vector are designated as pGEM(*ligB*var), pGEM(*ligB*con1) and pGEM(*ligB*con2).

Expression of rLigBvar protein in *E.coli*

The amplified PCR product of partial variable region of *ligB* gene (*ligB*var) was sub-cloned into pET33b vector at *NotI* sites. The *E.coli* TOP 10F' cells were transformed with the ligated mixture and the transformants were screened by kanamycin selection. The efficiency of transformation was 2 x 10^4/µg of DNA. The recombinant plasmid containing *ligB*var gene sequence was designated as pET33b (*ligB*var). In order to express rLigB var protein, BL21 strain of *E.coli* was used. *E.coli* BL21 cells were transformed with recombinant plasmids and grown on LB agar plates containing kanamycin (50µg/ml) at 37°C overnight. The transformed BL21 colonies were then screened for the presence of rLigBvar expressed protein. For that, individual colonies were grown in LB broth containing kanamycin (50µg/ml) at 37°C till the culture reached mid log phase or obtaining an OD_{600nm} of 0.4 – 0.5. The expression was induced at 30°C using 1mM isopropyl-β-D-thiogalactoside (IPTG). At this temperature, there would be increased expression and activity of a number of *E.coli* chaperons, thereby enhanced protein folding and reduced precipitation of inclusion bodies. Pre-induction incubation for 3 hr at 37°C was necessary to achieve mid-log phase growth. Samples were collected at 0, 1, 2, 3, 4, 5, 6, 7, 8 and 9 h post induction and were analyzed in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immuno-blot as per standard protocols.

Table 22. The details of LigB protein region targeted for expression study

LigB protein region designated in this study	Nucleotide position	Aminoacid position	Nucleotide length (bp)	Aminoacid length	Approximate molecular weight (kDa)
rLigBcon1	67-1935	23-645	1869	623	64.6
rLigBcon2	883-1935	295-645	1053	351	36.1
rLigBvar	1966-3096	656-1032	1131	377	39.3

Upon comparison of protein profiles of recombinant clones with that of control (vector and BL21 cells), MW size of ~47 kDa expressed protein was observed in case of recombinant clone, after 4h of induction. The intensity of bands increased gradually up to 8h post induction. In un-induced and control cultures, such specific bands were not observed even after prolonged incubation. The expressed truncated protein along with terminal fused His-tag was ~47 kDa, as observed in SDS-PAGE. Therefore, the bands at ~47 kDa correspond to cloned partial gene products (Fig 42A). The optimum time of harvest was 6h post induction (PI) and no much difference was observed in rate of expression either at 6 or 7 h PI but thereafter, there was a reduction in expression. This reduction could be attributed to autolysis of bacterial cells.

The proteins resolved in SDS-PAGE were transferred on to nitro cellulose membrane and were detected using positive serum (Serum from which leptospira has been isolated) (Fig. 42B). On analysis, specific band of ~47 kDa was observed in Western blot. This confirmed that the expressed recombinant proteins were specific to leptospira. The predicted size of the rLigBvar protein from amino acid (aa) sequences is about 39.3 kDa. The calculated size as per amino acids composition along with fused His-tag and as observed by the mobility in SDS-PAGE, is in agreement with the reported size indicating that the 47 kDa protein is the product from the cloned gene. Further work on the purification of the expressed proteins and its potential application as antigen in immuno-assay like ELISA for detection of leptospira antibodies in bovine samples is in progress.

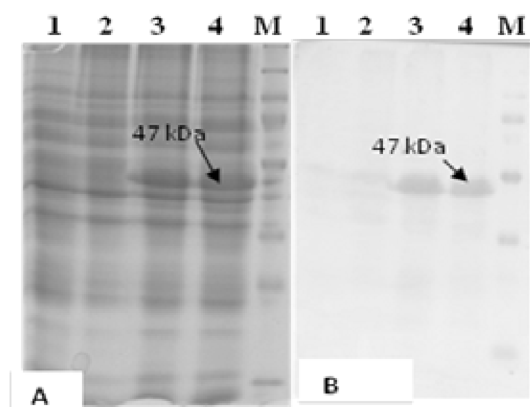


Fig. 42A) SDS-PAGE profile of recombinant LigB protein (rLigBvar); B) Western blot analysis for recombinant LigB protein (rLigBvar) Bacterial cell lysate from BL21 hostcell (Lane1); Vector pET33b control (Lane2); Recombinant LigBvar clone 1 and 2 (Lane3 & 4), respectively

Project : 11

Isolation, Identification and PCR based confirmation of Food-borne pathogens in Livestock and Livestock products

B. R. Shome and Rajeswari Shome

Listeria monocytogenes: Molecular analysis of genetic diversity

Information with regard to genetic diversity of *L. monocytogenes* strains prevalent in India is lacking The study was undertaken to generate data that would enable to determine the genetic relatedness between food and environmental *L. monocytogenes.* The relatedness of isolates was analyzed using the Sequence Type Analysis and Recombinational Test (START) software (pubmlst.org). Sequence types (ST) were clustered into clonal complexes or lineages. Based on the ratios of non-synonymous to synonymous polymorphisms (dN/dS ratios) for each locus and the index of association (*IA*) the tree of *L. monocytogenes* strains was constructed by the UPGMA. Sawyer's tests was run to provide evidence of recombinational exchanges of the intragenic sequences were also analyzed.

Global epidemiology of *L. monocytogenes*

From the sequences of the seven house keeping loci for each isolate, allelic profiles were assigned from MLST database (www.pasteur.fr/recherche/genopole/PF8/mlst/Lmono.html). The alleles defined for the MLST scheme were based on gene regions with sequence lengths of between 399 bp (*bglA*) and 537 bp (abcZ) (Fig. 43). For each locus the number of alleles ranged between 2 and 3. Multiple nucleotide sequence alignment did not show any significant insertions or deletions mutation in any of the intragenic fragments analyzed except for *ldh* in the clinical isolates LVCH08 and LCH09 which showed insertion of 6 nucleotides (position, 430 to 435bp), thus assigning them to ST 12. The ten isolates were resolved into ST7 (60%), ST12 (20%) and ST3 (20%). The two reference strains used were AL591824 EGD, FM242711-CLIP 80459. To analyze the population structure, ML trees were constructed from the concatenated sequences of the seven housekeeping loci of about 3288bp (3282 for *ldh*) (Fig. 44) and the topology showed that the 10 isolates were divided into two major clusters. Cluster

I included the serotype 1/2a isolates of ST 7 (n=6) and ST 12 (n=2) showing 67% similarity to the strain AL591824-EGD (ST 35) and the cluster II included the serotype 1/2b isolates of ST 3 (n=2) showing 100% similarity with the strain FM242711-CLIP80459 (ST 4).

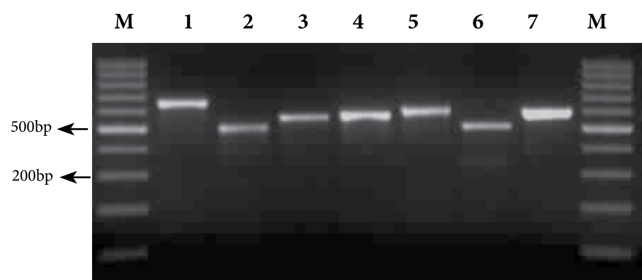


Fig. 43. Multi-Locus Sequence Typing (MLST) of seven house keeping genes of *L. monocytogenes*.Lane1 to 7: ABC transporter (*acbZ*, 607 bp), beta-glucosidase (*bgl*A, 458 bp), catalase (*cat*, 550 bp), Succinyl diaminopimelate desuccinylase (*dap*E, 523 bp), D-amino acid aminotransferase (*dat*, 557 bp), lactate deshydrogenase (*ldh*, 503 bp) and histidine kinase (*lhk*A, 592 bp) respectively; Lane M: 100 bp ladder (Fermentas).

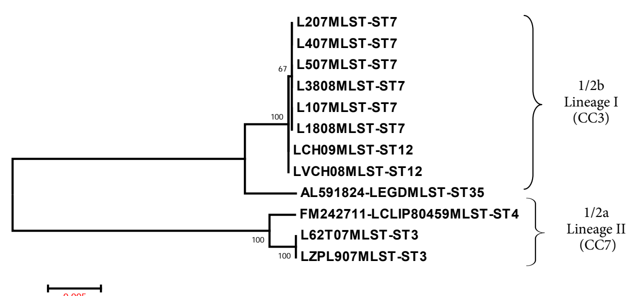


Fig. 44. Neighboring–joining tree of ten *L. monocytogenes* strains. The tree was constructed on the basis of the number of nucleotide differences in the seven house keeping gene fragments analyzed. Bootstrap values are shown at the interior branches of the node.

START2 analysis of the *L. monocytogenes* STs as of May 2010 (n=10 *vs* n=320) showed that dN/dS ratios of each locus was ranging from 0.000 (*bgl*A) to 0.0465 (*dat*) showing that they are not subject to positive selection. The G+C ratios for each locus ranged from 36.42 (*dat*) to 42.64 (*dap*E) and the proportion of polymorphic sites in each locus ranged from 2.506% (*bgl*A) to 12.101 % (*dat*) (Table 8). However, significant linkage disequilibrium was detected with I_A (0.2275, P<0.000) for MLST reference dataset of 320 STs and I_A (0.9062, P<0.000) for 10 STs of this study. A total of 32 possible CCs (groups) were identified following BURST analysis and predicted that ST-3 was the ancestor of lineage I (CC3) and ST 7 to be the ancestor of lineage II (CC7). It was found that the isolates belonging to ST 3 were having 18 SLV and 2 DLV, while isolates belonging to ST 7 and 12 were having 15 and 13 SLV and 2 and 4 DLV respectivley. ST 7 complex dominated the population and contained 60% of isolates with 17 STs as its descent.

The BURST analysis revealed that there was one major lineage within this collection of isolates (the ST-7 clonal complex) which contained 34 STs with ST-12 as a single locus variant of ST-7, which suggests it may be well fitted to listerial infection. The value of the I_A indicated significant linkage disequilibrium among the alleles at each of the seven housekeeping loci (*P*=0.01). However, in the present study, no evidence for linkage was detected when the analysis was performed separately in serotype 1/2a isolates and serotype 1/2b isolates idicating that the recombination should be rare between strains belonging to different genetic lineages. These results suggested the hypothesis that most of the clinical cases are caused by only a few clones that either are particularly common in the environment or have an unusually high level of pathogenicity. In the epidemiological context, MLST could be a useful tool for the surveillance systems for listeriosis allowing the identification and the distribution of these *L. monocytogenes* clones in the environment. The extension of the present analysis to a higher number of isolates could contribute to a better knowledge of the structure of the *L. monocytogenes* population.

Local epidemiology of *L. monocytogenes*

For Multi Virulence Locus Sequence Typing, partial coding sequences (CDs) of the select seven virulence genes (*hlyA*, *inlA*, *inlB*, *ami*, *iap/p60*, *plcA* and *plcB*) sequenced and analyzed are diversely located in the *L. monocytogenes* genome in strain EGD-e (Fig. 45; Table 23). Different allelic sequences (with at least a one-nucleotide difference) were assigned arbitrary numbers within the isolates with respect to the reference sequences from BLAST program. Multiple sequence alignment showed significant 3 nucleotides deletion in *iap* of L62T07 and LZPL907 isolates which led to 56th amino acid deletion. The allelic combination of all the seven loci clearly grouped the isolates into three major clusters. New alleles have been reported for the first time in *hlyA* locus of L407 and *ami* locus of L62T07 and LZPL907. The percentages of polymorphic nucleotide sites ranged from 3.629% for *hly*A to 14.062% for *iap* (Table 23). Although non-synonymous substitutions were found in all six loci, most of the nucleotide polymorphisms resulted

Table 23. Allelic polymorphisms in the gene fragments analyzed.

Gene	Size (bp) of fragments analyzed	Coverage of complete CDS (%)	Allelic location (nt) in strain EGD	No. of alleles	No. of polymorphic sites	% of polymorphic sites	Mean GC content (%)	d*N*/d*S* ratio
Housekeeping loci								
*abc*Z	537	30.29	109530–110066	2	16	2.979	37.80	0.0178
*bgl*A	399	28.18	144181–144579	2	10	2.506	40.50	0.0000
cat	486	33.13	153619–153134	2	18	3.703	41.11	0.0152
*dap*E	462	40.53	87940–88401	2	32	6.926	42.64	0.0447
dat	471	54.14	11894–12364	2	57	12.101	36.52	0.0465
ldh	453	48.09	14780–15232	3	19	4.194	41.97	0.0365
*lhk*A	480	33.33	128780–129259	2	15	3.125	37.33	0.0411
Virulence loci								
*hly*A	496	31.19	6691–7186	3	18	3.629	35.32	0.0148
*inl*A	487	25.25	97044–97521	2	35	7.186	40.22	0.0907
*inl*B	478	25.25	97044–97521	2	18	3.765	31.35	0.2047
ami	526	19.09	240347–240872	2	73	13.878	37.40	0.0510
iap	576	39.75	259761–260336	2	81	14.062	39.83	0.1837
*plc*A	513	53.77	4908–5420	2	34	6.627	37.04	0.1992
*plc*B	502	57.70	11697–12198	2	37	7.370	34.44	0.0755

Table 24. Amino acid substitution sites in each of the virulence gene fragments with reference to AL591974/NC003210-EGD strain.

Isolate ID	*hly*A		*inl*A									*inl*B								
	69 D	147 V	40 A	44 A	50 A	78 T	98 S	100 P	124 V	154 H		33 P	41 S	61 A	65 N	83 V	109 A	124 V	130 L	156 P
L1	.	.	.	.	.	A	.	.	A	.		S	P	.	.	.	T	I	.	.
L2	.	.	.	.	.	A	.	.	A	.		S	P	.	.	.	T	I	.	.
L4	G	.	.	.	.	A	.	.	A	.		S	P	.	.	.	T	I	.	.
L5	.	.	.	.	.	A	.	.	A	.		S	P	.	.	.	T	I	.	.
L18	.	.	.	.	.	A	.	.	A	.		S	P	.	.	.	T	I	.	.
L38	.	.	.	.	.	A	.	.	A	.		S	P	.	.	.	T	I	.	.
KAR	.	.	.	.	.	A	.	.	A	.		S	P	.	.	.	T	I	.	.
CH	.	.	.	.	.	A	.	.	A	.		S	P	.	.	.	T	I	.	.
62TLSA	.	I	E	P	V	A	N	S	A	Y		.	P	L	S	I	A	I	.	L
ZPL9	.	I	E	P	V	A	N	S	A	Y		.	P	L	S	I	A	I	.	L
CLIP80459	.	I	E	P	V	A	N	S	A	Y		S	P	L	.	.	A	I	I	.

Isolate ID	*iap*									*plc*A						*plc*B							*ami*
	56 N	77 S	99 T	106 H	114 K	127 A	128 V	150 A	179 V	4 N	23 I	59 N	66 F	158 R	167 P	2 K	35 R	59 T	124 A	125 K	130 D	134 D	X
L1	.	T	.	.	.	.	.	V	I	.	M	K	Y	.	.	.	.	.	.	.	.	.	X
L2	.	T	.	.	.	.	.	V	I	.	M	K	Y	.	.	.	.	.	.	.	.	.	X
L4	.	T	.	.	.	.	.	V	I	.	M	K	Y	.	.	.	.	.	.	.	.	.	X
L5	.	T	.	.	.	.	.	V	I	.	M	K	Y	.	.	.	.	.	.	.	.	.	X
L18	.	T	.	.	.	.	.	V	I	.	M	K	Y	.	.	.	.	.	.	.	.	.	X
L38	.	T	.	.	.	.	.	V	I	.	M	K	Y	.	.	.	.	.	.	.	.	.	
KAR	.	T	.	.	.	.	.	V	I	.	M	K	Y	.	.	.	.	.	.	.	.	.	
CH	.	T	.	.	.	.	.	V	I	.	M	K	Y	.	.	.	.	.	.	.	.	.	
62TLSA	-	.	S	N	E	V	A	.	.	S	M	K	.	H	S	N	K	A	V	.	.	E	
ZPL9	-	.	S	N	E	V	A	.	.	S	M	K	.	H	S	N	K	A	V	.	.	E	
CLIP80459	-	.	S	N	E	V	A	.	.	S	M	K	.	H	S	N	K	A	V	Q	N	E	

Synonymous site. - Deletion. X No olymorphism noticed.

in synonymous substitutions and is depicted with reference to strains AL591974/NC003210-EGD and FM242711-CLIP 80459. No amino acid substitution was observed in *ami* alleles, while 2, 8, 9, 9, 6 & 7 amino acid substitutions were observed in *hlyA, inlA, inlB, iap, plcA* and *plcB* alleles respectively (Table 24). ML trees were constructed form the concatenated sequences of the seven virulence loci analyzed (3578bp, 3575 bp in *iap*) revealed two major clusters (Fig. 46). Cluster I included serotype 1/2a ST 7 and ST12 isolates showing 100% similarity to the strain AL591974/NC003210-EGD and the cluster II included the serotype 1/2b isolates of ST 3 showing 100% similarity with the strain FM242711-CLIP80459. START2 analysis revealed the dN/dS ratios of each locus were ranging from 0.0148 (*hly*A) to 0.2047 (*inl*B). The G+C ratios for each locus ranged from 31.35% (*inl*B) to 40.22% (*inl*A) and the proportion of polymorphic sites in each locus ranged from 3.629% (*hly*A) to 14.062 % (*iap*) (Table 24). However, significant linkage disequilibrium was detected with I_A (0.4045, P<0.000).

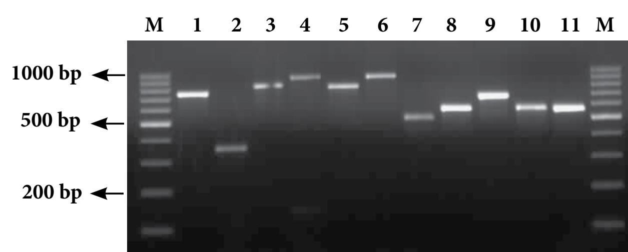


Fig. 45. Multi-Virulence-Locus Sequence Typing of seven virulence genes of *L. monocytogenes.* Lane 1 to 11: hemolysin (*hly*A1, 746 bp), hemolysin (*hly*A2, 356 bp), hemolysin (*hly*A3, 825 bp), internalin A (*inl*A1, 928 bp), internalin A (*inl*A2, 791bp), internalin A (*inl*A3, 910 bp), internalin B (*inl*B, 509 bp), autolysin amidase (*ami*, 571 bp), invasion associated protein (*iap*, 675 bp), phospholipase A (*plc*A, 566 bp) and phospholipase B (*plc*B, 562 bp) respectively; Lane M: 100 bp ladder (Fermentas).

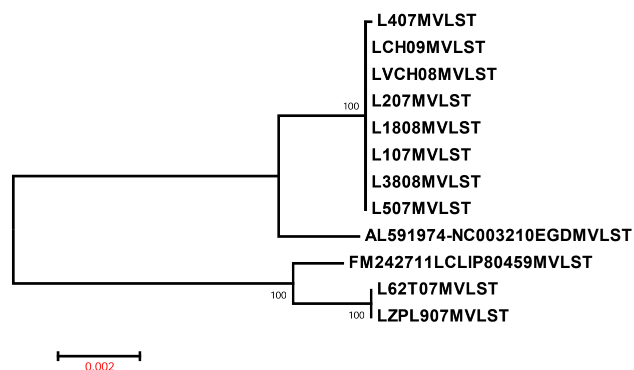


Fig. 46 : Neighboring–joining tree of 10 *L. monocytogenes* strains. The tree was constructed on the basis of the number of nucleotide differences in the seven virulence gene fragments analyzed. Bootstrap values are shown at the interior branches of the node.

The percentage of nucleotide polymorphisms in the housekeeping genes ranged from 2.506 to 12.101% against 3.629 to 14.062% in virulence genes. Though the allelic profiles of both housekeeping and virulence loci showed three major clusters, the grouping was different. As PCR amplification and DNA sequencing become increasingly automated and commercially available, MVLST may provide a more convenient tool for studying epidemiology of *L. monocytogenes* than fragment-based typing methods. More extensive MVLST analyses conducted on the basis of the investigation of a larger number of strains may provide critical validation of the present study and informative clues for the study of the virulence genes and pathogenesis of *L. monocytogenes*.

In conclusion, we have adopted the classical MLST and MVLST scheme for a major pathogen of zoonotic importance, *L. monocytogenes*, and demonstrated its potential for future studies of global and local epidemiology and population biology. The data generated from the *L. monocytogenes* isolates in the present study provides a framework upon which the distribution of genes involved in pathogenicity can be superimposed and that can be expanded in future studies to improve understanding of the population structure and global epidemiology of this pathogen

Project : 12

Molecular diagnostics for the detection of carrier status of Surra

P.P. Sengupta, V. Balamurugan and K. Prabhudas

Trypanosoma evansi, a haemo flagellate protozoan parasite, causes surra or trypansomiasis in wide range of species including cattle, buffalo, canines, felines, camels, equines etc. The disease is transmitted by tabanid flies and characterized by high mortality and morbidity. The clinical cases of trypanosomiasis can be easily diagnosed by conventional Giemsa's stained blood smear method. However, the detection of carrier status is a challenge under the field conditions. When the immunity in the host gets declined, the parasite takes upper hand and the disease flares up. Thus the carrier animals become nuclei for propagation of the disease in a particular area. Hence the detection of the carrier animal is very important to eradicate the disease. Further, it is already known that variable

surface glycoprotein (VSG) which possesses diagnostic importance as it is expressed in early, middle and late stage of the infection. Thus it is helpful for detection in acute and chronic stages of infection.

Investigation

An investigation team comprising of scientists from IAH&VB and PD_ADMAS visited Bannerghatta National Park to investigate the cause of death of several tigers and collected the blood samples from 11 ailing tigers. All the samples showed negative reaction in PCR with DITRY F/R primers. Apart from this, blood samples from 29 cattle and 9 buffalo with history of chronic fever and weakness were collected from Davangere District of Karnataka and subjected for PCR and mouse inoculation test. 3 cattle samples showed positive reaction with PCR but mouse could not pick up the infection.

RT-PCR

At the peak parasitaemia the blood was harvested from the experimentally infected rats. Later the tryps were purified from blood cells by passing through DEAE cellulose column chromatography. The mRNA from the purified *Trypanosoma evansi* was isolated using RNeasy kit. The isolated mRNA was subjected to Reverse Transcription PCR (RT-PCR)., using nanomer primer for cDNA synthesis. The cDNA synthesized was subjected to PCR by high fidelity PCR enzyme mix (long PCR mix), using the primer pairs – TEVSG F and TEVSG R. The amplified product (1.5kb) was purified from the agarose gel using gel extraction kit.

Cloning of VSG gene

VSG gene isolated from the gel was cloned into pGEMT (TA cloning vector) using, T4 DNA ligase enzyme. Then the ligated product was transformed into *E.coli* (Top 10) cloning host and recombinant clones were screened and selected for further use. Then, VSG gene from pGEMT was released using *NotI* single digestion and ligated into pET33b (linearised using *NotI*). The pET33b with VSG (*Not* I) was transformed into *E.coli* (Top10) cloning host. The recombinant clones were selected using kanamycin (50µg/ml) and preserved in cryopreserved glycerol stock for further use.

Expression study

pET33b with VSG (*Not* I) plasmid DNA was isolated from *E.coli* (Top10) and transformed into BL21 (expression host) for expression. Recombinant BL21 clones were selected and induced to express VSG *(NotI)* gene using IPTG (1mM final concentration). The induction process was carried out up to 7 hours and maximum expression was observed after 6 hours of induction. SDS-PAGE analysis of the non-induced transformed BL21 cell line and induced transformed BL21 cell lines at different intervals were done. It showed that after 6 hours the expression level was maximum (Fig. 47). The induced protein was purified by NiNTA agarose gel filtration. The size of the recombinant protein was 43.8 kDa. The recombinant protein was characterized by Western blotting. Western blot analysis showed that the recombinant protein was immune reactive with the rabbit hyperimmune sera raised against buffalo, dog, leopard and lion isolates of *T. evansi*, but the immunoreactivity was maximum against the buffalo isolate hyperimmune sera (Fig. 48). The immunoreactivity of the expressed protein with the rabbit hyperimmune sera raised against buffalo and canine isolates were significant.

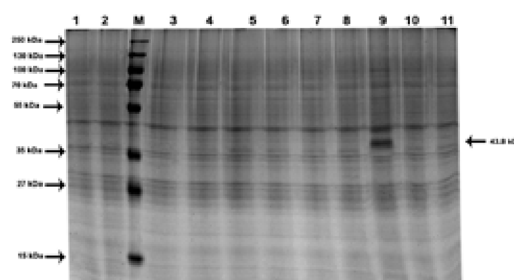


Fig. 47. SDS PAGE analysis of recombinant VSG *(NotI)* protein, Lane 1: BL 21 with pET33b Lane 2 : BL 21 Lane M: protein marker, Lane 3: uninduced BL21 with pET33b and insert of VSG, Lane 4-11: protein expressed by Induced VSG (NotI) protein at different time intervals starting from 1st hour to 8th hour.

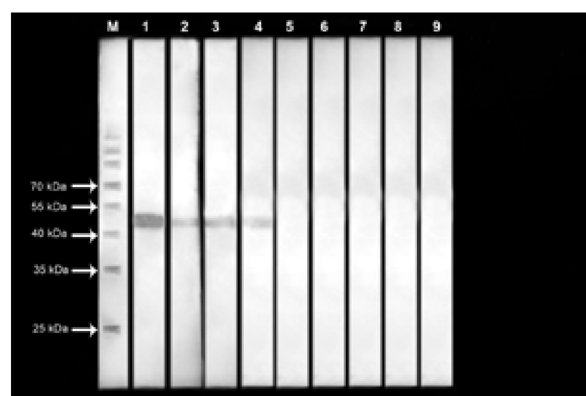


Fig. 48. Western blot analysis of recombinant VSG (*Not* I) recombinant protein with rabbit hyper immune sera raised against different isolates of *T.evansi*. Lane M : Prestained protein marker Lane 1-4 shows the reaction of Induced recombinant protein VSG (*Not* I) vs rabbit hyperimmune sera raised against buffalo, dog, lion and leopard isolates of *T. evansi*, respectively. Lane 5-8 shows the reaction of uninduced recombinant protein VSG (*Not* I) vs. rabbit hyperimmune sera raised against buffalo, dog, lion & leopard isolates of *T. evansi*, respectively. Lane 9: shows the reaction of Induced recombinant protein VSG (*Not*I) vs. rabbit healthy control sera.

Project : 13

Comparative virulence of *Trypanosoma evansi* infection in rodent model

P. Krishnamoorthy, P.P. Sengupta, M.R. Gajendragad and K. Prabhudas

The various isolates of *Trypanosoma evansi* (canine, buffalo, leopard, lion) available at parasitology laboratory, PD_ADMAS, Bengaluru were used in the study. Induction of experimental infection of trypanosomes in Wistar albino rat by giving 0.2ml containing $1x10^5$ trypanosomes per ml intraperitoneally. Sequentially collection of blood, serum and tissues (liver, lung, spleen, heart, kidney, adrenal, abdominal muscle and testis) were collected from day one to day seven post infection. Counting of trypanosomes in blood using haemocytometer method was done. Histopathological studies revealed trypanosomes in chamber of heart muscle (Fig. 49); lung showed focal infiltration of plasma cells and mononuclear cells (Fig. 50); testis showed interstitial edema (Fig. 51). The virulence pattern of different isolates was compared.

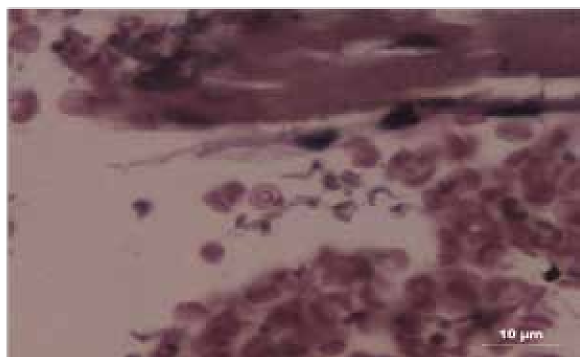


Fig. 49. Heart - *Trypanosoma evansi* (buffalo isolate) (H&E, Scale bar=10μm)

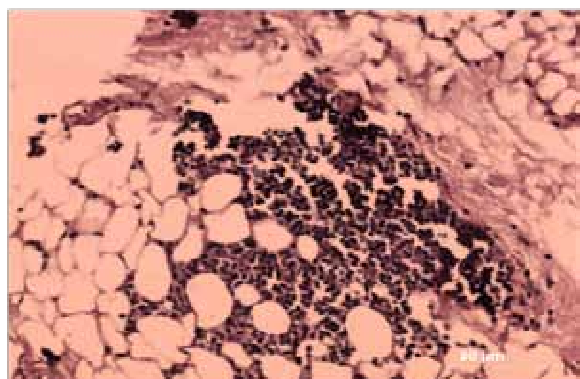


Fig. 50. Lung showing focal mononuclear cell infiltration (H&E, Scale bar=50μm)

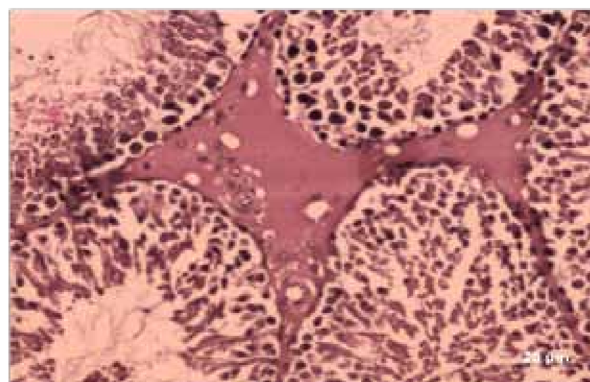


Fig. 51. Testis showing edema in the interstitial space (H&E, Scale bar=20μm)

Project: 14

Population epidemiology of livestock diseases

National livestock serum bank facility

M. R. Gajendragad

The National livestock serum repository is being used to study the population epidemiology. The serum samples collected from various species by stratified random sampling method are deposited at the repository by the collaborating units of the directorate. During the year 2010-11 a total of 2466 serum samples from eight livestock species were received. The breakup of the serum received at the repository is given in the pie diagram (Fig. 52). Each of the serum samples received at the bank was assigned a bar code and entered in the serum bank database. As part of the study, 1085 cattle serum samples referred to the directorate for either screening or confirmation of brucellosis were subjected to AB-ELISA and 67% of them were found to be positive for the antibodies.

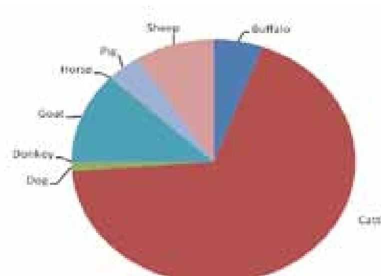


Fig. 52. Serum receipt at the Serum Bank from April 2010 to March 2011

Project: 15

Epidemiological significance of coagulase negative Staphylococcus of diverse genetic background isolated from bovine sub-clinical mastitis

Jagadish Hiremath, P. Krishnamoorthy and B. R. Shome

Mastitis remains a major challenge to the worldwide dairy industry despite the widespread implementation of mastitis control strategies. In addition to *Staphylococcus aureus*, the importance of different coagulase negative staphylococci (CNS) causing bovine mastitis has significantly increased in recent years. The molecular sub-typing and virulence characterization of CNS is required to understand the epidemiological importance of the isolates in causing clinical or subclinical mastitis. Hence the proposed RPF is aimed at understanding the epidemiological significance of coagulase negative staphylococcus of diverse genetic background isolated from bovine subclinical mastitis and comparing them with those isolated from other organ system. The CNS will be isolated and identified from bovine milk, faecal sample, urogenital sample and skin. Depending upon the type of species they will be subjected to genotyping methods like multilocus sequence typing (MLST), pulse-field gel electrophoresis and *spa* typing to identify the dominant strain prevailing in the region. The strains of different organ system will compared to establish niche specificity. The study will also focus on phenotypic and genotypic virulence analysis of the isolates. The prototype organism will be subjected to pathogenicity test in mice. The results of this work and their analysis lead to overall understanding of the CNS prevalence, genotype, and their virulence potential. Further, this information will help in effective disease control and prevention of bovine mastitis.

Sample Collection: A total of 60 samples, 30 each of urogenital and skin swabs were collected into brain heart infusion broth (BHI) from the lactating animals from unorganized dairy sector in Bellur village, Bangalore, Karnataka.

Isolation and phenotypic identification of Staphylococcus: The overnight incubated samples in BHI were streaked in to *Staphylococcus*110 medium and incubated at 37 °C for 24 h. After 24 h of incubation, the colonies of each isolated microbial strains suspected to be *Staphylococcus* were streaked on BHI agar to obtain the pure cultures and presumptively identified as *Staphylococcus* on the basis of colony morphology, pigmentation, Gram staining, cellular morphology, catalase and oxidase activities. Out of 60 samples, 108 isolates were presumed to be staphylococcus and are need to be further characterised by PCR to find out the species and subspecies.

Genotypic Identification of Staphylococcus

The process of identification to species and subspecies level is in progress. Of the 108 samples 11 samples were subjected to DNA extraction by boiling method which was followed by staphylococcus genus specific PCR. The result showed that out of 11, two were positive for staphylococcus genus with product size of 842bp, where as rest were negative.

External Funded Projects

Project: 16

All India Network Project on Bluetongue

Divakar Hemadri and Mudassar Chanda

Introduction and disease status for the year 2011 in Karnataka

Bluetongue (BT) is an arthropod-borne viral disease primarily of sheep caused by the bluetongue virus (BTV), which is the prototype species of the genus *Orbivirus* in the *Reoviridae* family. The virus can also infect many domestic and wild ruminants such as goat, cattle, buffalo, camel, deer and other artiodactyls. The virus is transmitted between hosts by certain species of biting midges (*Culicoides* species), which are most abundant and active in hot and humid climates. The first outbreak of BT in India was reported in the early 1960s and thereafter regular outbreaks have been recorded in different parts of the country. Currently BT is endemic in India. In order to have concerted research effort on Bluetongue, All India Network on Bluetongue was initiated in 2001. The Project Directorate on ADMAS was included in this programme as non funded centre in the year 2009 and the research progress under this programme is given below.

During the period under report, the animal disease surveillance scheme of the Karnataka state has recorded 73 outbreak of Bluetongue disease in sheep/goats in six districts of the state, *viz.*, Tumkur, Koppal, Chitradurga, Davanagere, Haveri and Chikkaballapur. In addition to the above districts the outbreak information of suspected BT were received by the ADMAS team directly from field veterinarians of Bidar, Raichur and Gadag districts. A quick look at the trend line (Fig. 53) indicates a marginal decrease

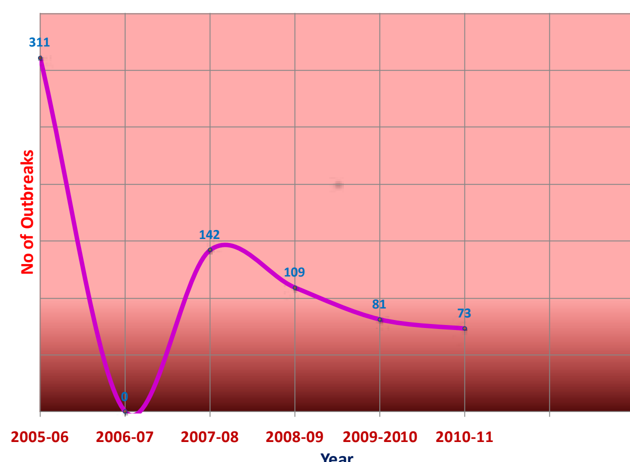


Fig. 53. Bluetongue outbreaks in Karnataka 2005-11

in the number of bluetongue outbreaks in the state compared to the previous year. Interestingly, the number of outbreaks consistantly less than previous years from 2007-08 to 2011. The reason for the above observation needs to be explored.

Whatever may be the reason for the above observation, the first outbreak of the season was in July 2010, in Sira taluk of Tumkur, where in six sheep of L.H.Palya and Jodidevarahalli villages were affected and the disease subsided without further spread. The reason for such behaviour is difficult to explain from the existing data, nevertheless the adverse effect of rainy season and other environmental factors on the survival of the main vector, culicoides cannot be ruled out. After two months gap, the disease was reported from northern districts of Raichur, Koppal and Bidar. Since these districts border Andhra Pradesh and it is very tempting to speculate that the disease was probably spread from the said state, as it had suffered bluetongue outbreaks in September and October. Further molecular epidemiological analysis and serotyping of the virus from clinical samples may resolve this issue. In November, the disease was reported from southern districts like Chitradurga and Tumkur. In all 19 outbreaks involving four taluks of the Chitradurga district were reported besides the Sira taluk of Tumkur district which reported three BT outbreaks. The disease remained active in Chitradurga district in the month of December and so also is Gauribidanur taluk of Chikkaballapur district. The disease was in full flow in the month of January in Chikkaballapur district as two more taluks (Siddlaghatta and Chintamani) reported outbreaks whereas decrease in number of outbreaks in Chitradurga and Tumkur was observed. Comparison of disease outbreak profile (Fig. 54) over the past five years indicates higher incidence during winter months of November and December, however, this year highest number of outbreaks were reported in the month of January. This year outbreak curve had two smaller peaks and broader base compared to sharper peaks in the previous years (Fig. 54). This is probably due to less wide spread disease incidence during a given month, which can be seen from the outbreak maps (Fig. 55)

Field Epidemiology

During the period under report, the BT team of the PD_ADMAS (Fig. 55) attended the suspected bluetongue outbreaks in Gadag (Hulkoti and Chikkondi), Bidar (Bakchaudi and Kolar), Chitradurga (Madakaripura, Dandina Kurubara Hatti, Hampayyana Malige Gollara Hatti, Belaghatta, Haikal,

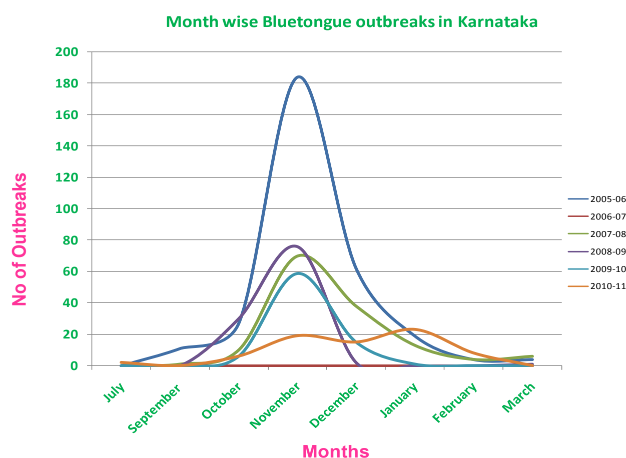


Fig. 54. Year wise and month wise Bluetongue outbreaks in Karnataka

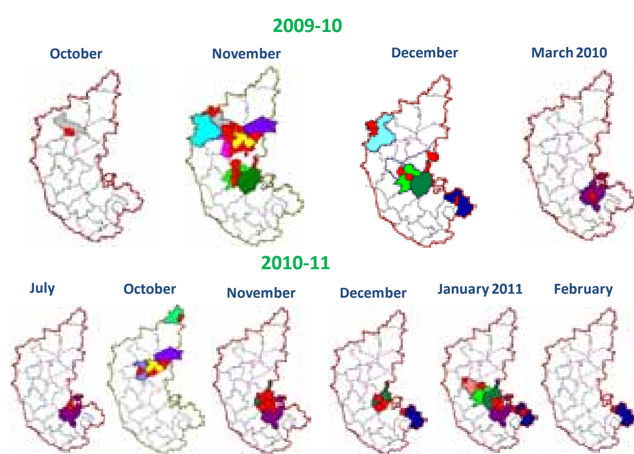


Fig. 55. BT outbreak maps for the year 2009-10 & 2010-11
The affected districts are shown in various colours whereas all the affected taluks in Red

Fig. 56. Satellite image showing the places of Bluetongue outbreaks in Chitradurga Taluk

Bommakkanahatti, Bettada nagenahalli in Chitradurga Taluk, Govt Sheep Farm, Nayakana Hatti in Challakere taluk), Chikkaballapura (Gundugere, Irgampalli of Siddlaghatta taluk and Narayanahalli of Chintamani Taluk) and Raichur (few villages in Sindhanoor taluk). The places of outbreaks in Chitradurga taluk (attended by ADMAS team) are shown on the Map (Fig. 56).

During the field visits various clinical samples and epidemiological parameters such as clinical signs, flock size, morbidity, mortality, species/ breed of animal affected, possible source of infection etc were recorded. Besides the epidemiological parameters geographical coordinates of the place of outbreak was also collected. The detail list of samples collected and the case fatality rates are given in Table 25 and 26.

In the villages surrounding Chintamani and few villages in Chitradurga taluk, there was history of recent introduction of new animals from other places. In one flock (Dandina Kurubara Hatti) the animals were brought from a village near Chintamani (Chikkaballapur District). Incidentally both places have reported the disease almost at the same time and it is yet to be established whether these outbreaks were connected or not and so are the outbreaks in Andhra and Karnataka. This is important considering substantial migration of sheep flocks occurs, particularly in Northern Karnataka from one district to other or even between states.

Spatial Epidemiology

The work on spatial epidemiology of Bluetongue has been recently initiated at PD_ADMAS. As a part of the work, creating taluk level digital maps for Karnataka has been initiated. A database of Bluetongue disease outbreak has been created. Procurement of meteorological data, animal census data, soil data and maps, land cover and land use etc are in progress. However, with existing facilities and the data, an attempt was made to study the role of ecological factors if any on the occurrence of the disease by overlaying various maps.

Extraction of weather parameters from the places of out break

Ecological factors play an important role in outcome of many diseases and to study this, a village Irakalgudda, (Fig. 57) in Koppal district was selected and based on geographic coordinates of this village, weather parameters such as precipitation, wind speed,

Table 25. Details of sample collected during 2010-11

S.No.	Place of collection	No. of samples collected
1.	Kamthana (Bidar District)	10
2.	Bakchaudi (Bidar District)	15
3.	Chintamani and surrounding villages (Kolar district)	18
4.	Chitradurga and surrounding villages	30
	Total	**73**

Table 26. Case fatality rates at different villages

S.No	Name of the village	No. of animals affected	No. of animals dead	Total Number of animals in flock	CFR (case fatality rate)
1.	Madakaripura	30	15	100	50%
2.	Dandinakurubara hatti	20	04	95	20%
3.	Hampayanamalige Gollara hatti	300	100	800	33.3%
4.	Belaghatta farm	18	06	100	33.3%
5.	Haikal	100	50	400	50%
6.	Bommakana hatti	40	06	300	15%
7.	Challakeri farm	60	10	400	16.6%
8.	Bettadanagena halli	60	04	400	6.6%
9.	Gundugere	10	5	80	50%
10.	Guttar chindope	05	01	20	20%
11.	Irgampalli	10	02	100	20%

temperatures were extracted at every three hours interval and monthly averages were calculated.

Comparison of the various ecological factors such as mean temperature, precipitation, wind speed etc over the past two years is shown in the Figs. 58 & 59. A quick look at the graphs indicate that average monthly mean temperature for the last two years have remained almost identical where as mean precipitation for the months of August, September and October during the year 2009 was about two times more than that was for the same period in 2010. It has been reported that many places of Bagalkot, Raichur, Bellary and Koppal district were reeling under floods during that time. Overlaying of district map over the layer of water lines and rivers (Fig. 60) indicates that many places where the outbreaks were recorded actually fall in those areas near water lines and rivers. In this context, the moist soil after the floods coupled with ideal wind speed (Fig. 61) may have helped large scale breeding of bluetongue vector Culicoides species thereby enhancing the chances disease outbreak and spread. Unfortunately, the present analysis is constrained by lack of statistical analysis due to want of large scale digital maps of water lines and rivers, soil type, land cover and land use etc. Nevertheless the present work which is initiated recently envisages procuring those maps in the coming

Fig. 57. GPS recording and map of the place of outbreak (koppal Taluka). The weather parameters were recorded for this place

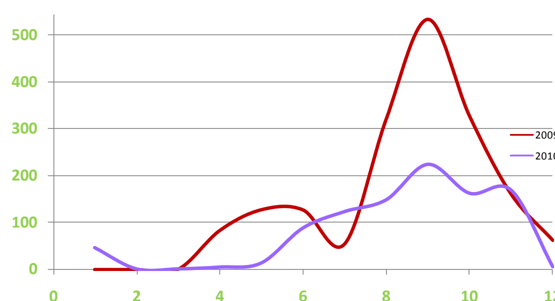


Fig. 58. Comparison of sum of precipitation during the years 2009 and 2010

days.

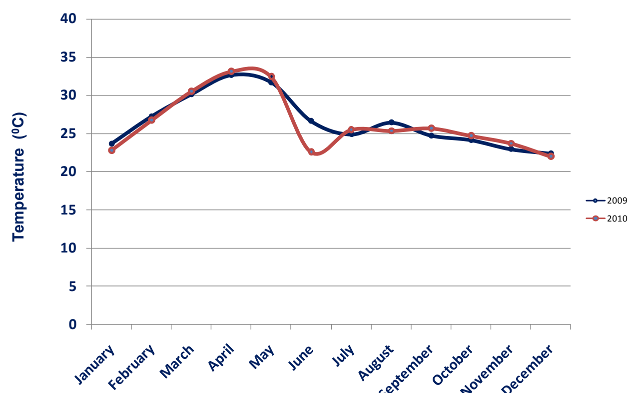


Fig. 59 : Comparison of average monthly temperature during the year 2009 and 2010

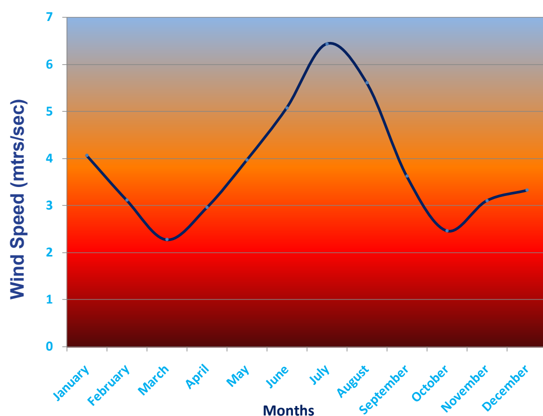


Fig. 61 : Average Monthly wind speed during the year 2009

Fig. 60. Water line & rever map of Bagalkot, Raichur, Koppal, Bellary and Gadag Dist.

Molecular Epidemiology

Molecular epidemiological work based on clinical samples, which was initiated a year back is continuing. The clinical samples collected from suspected bluetongue outbreaks from Koppal, Sindhanoor, Bagalkot districts were positive for Bluetongue virus by NSP gene PCR. In order to identify the serotype involved, indigenously designed primers were used in a multiplex PCR format, where in four reactions were used to rule out/detect 11 bluetongue serotypes. The prilimimary analysis indicated that clinical samples

Table 27 : Details of the sheep serum samples screened against bluetongue

State	District	No of samples	Positive	Negative	Doubtful
Andhra Pradesh	Guntur	20	2	17	1
Gujarat	Banaskantha	19	0	19	0
	Vadodara	47	0	47	0
	Baruch	20	5	14	1
	Narmada	10	2	8	0
	Navasari	12	0	12	0
	Surendranagar	17	6	11	0
	Valsad	67	15	50	2
	Rajkot	103	72	27	3
	Jamnagar	73	32	38	3
	Anand	9	6	3	0
	Kheda	15	2	10	3
Madhya Pradesh	Betul	8	2	6	0
	Sagar	40	6	32	2
	Shivapuri	24	0	21	3
Karnataka	Mandya	150	25	120	5
	Kolar	43	10	30	8
Grand Total		**677**	**185**	**465**	**26**

collected from Bagalkot district were positive for serotype 1 and those obtained from Sindhanoor were positive for serotype 2 and 1. Further fine tuning of these PCR for routine use and sequencing the PCR products are under process. It is worth mentioning that identification of two serotypes from neighbouring flocks substantiated the migratory nature of these flocks.

Sero-epidemiology

Under this work 677 serum samples (Table 27) either collected/received from Karnataka, Madhya Pradesh, Gujarat, Andhra Pradesh were screened for the presence of bluetongue antibodies using indirect sandwich ELISA developed at IVRI Mukteswar. Analysis showed that 185 samples (27.32%) were positive for anti-BTV antibodies. Most of the samples showed seropositivity around 20-30%, however, the samples from Rajkot (72/103, 69.90%), Jamnagar (32/73, 43.83%), Anand (6/9, 66.67%) showed higher positivity indicating probable recent infection.

Project: 17

ICAR: Outreach Programme on Zoonotic Diseases

Rajeswari Shome, V. Balamurugan and M. Nagalingam

PD_ADMAS is one of the twelve centres which have been actively involved in working on zoonotic diseases under Outreach Programme on Zoonotic Diseases (OPZD) by Indian Council of Agricultural Research (ICAR) since November, 2009. The components allotted were three zoonotic diseases namely brucellosis, leptospirosis and listeriosis with the following objectives.

1. To monitor the burden of brucellosis / leptospirosis / listeriosis in livestock including pets.
2. To determine the incidence of active infections among the human with particular reference to risk groups.
3. To detect the antigen/antibody in milk/meat and their products.
4. To generate molecular data of the different pathogens.
5. The epidemiological analysis of disease in livestock /pet species and human.

1. Monitoring the disease burden in livestock

For monitoring all the three diseases, random sampling and clinical samples from suspected animals to record active infection were carried out.

(A) Brucellosis

The serum samples collected from livestock were subjected to various diagnostic tests such as Rose Bengal Plate test (RBPT), Enzyme Linked Immuno Sorbent Assay (ELISA) and Polymerase Chain Reaction (PCR) (Table 28). Although RBPT and ELISA can reveal the exposure of the particular animal to *Brucella*, PCR can confirm l the current infection status.

Table 28. Livestock samples analyzed for brucellosis during 2010-11

Species \ No.	Number of samples	RBPT Positive	ELISA Positive	PCR Positive
Cattle	261	11	20	0
Horse	51	1	1	0
Pig	39	20	29	8
Sheep and goat	106	1	3	0
Total samples analysed	457	33	53	8

Among cattle samples screened, 4.21% and 7.66% samples were positive by RBPT and ELISA, respectively. None of the cattle samples were PCR positive. Similarly in equine samples, only one sample was positive by both RBPT and ELISA. Interestingly, among pig samples **51.2%, 74.35%** and **20.51%** samples were positive by RBPT, ELISA and PCR, respectively indicating the higher prevalence of both antibody and antigen in pig samples. The study revealed brucellosis as cause of abortion in the pig farm. In case of sheep and goat, the result indicated the negligible prevalence of the disease.

(B) Leptospirosis

For monitoring leptospirosis, isolation was carried out in EMJH medium and fortnight observation under dark field microscope till two months were carried out to rule out negative for isolation. Standardized PCR techniques involving *16s rRNA*, *secY* and *rpoB* genes were also carried out. A total of 437 samples were subjected to various assays and the results were summarized in Table 29.

Table 29. Clinical samples analyzed for leptospirosis during 2010-11

Species \ No.	No. of Samples subjected to isolation	Isolation positive	No. of Samples subjected to PCR	PCR Positive
Cattle	228	9	125	55
Buffalo	34	5	3	-
Sheep	89	-	--	--
Goat	13	-	16	-
Pig	30	3	36	22
Dog	--	--	7	1
Horse/donkey	32	1	29	9
Tiger	11	-	11	-
Total	437	18	227	87

(C) Listeriosis

Serum samples from cattle (n=10) and goat (n=16) received from Maharashtra state were screened for listeriosis by extracting DNA and subjecting to species-specific PCR. Four out of 10 cattle and 11 out of 16 sera samples were positive for *Listeria monocytogens* species specific PCR. All the samples were processed for isolation by inoculating sera samples initially in UVM-1 for 24 hrs at 30 ^{0}C, followed by in UVM-2 for 24 hrs at 37 ^{0}C and finally cultured on PALCAM agar. The characteristic listerial growth could be observed on PALCAM agar in one each of buffalo and goat sample on cold enrichment procedure for 15 days.

Since indigenously available ELISA kits were not available, sero monitoring of listeriosis on wide scale was not possible and hence development of an ELISA kit was planned.

Two standard strains of *Listeria monocytogens* (1/2a and 4b) were procured, confirmed bacteriologically and also by genus and species-specific PCR assays. The confirmed strains were used for the testing best production of listeriolysin 'O' at different time intervals (6, 12, 18, 24 hrs) in two different broths (brain heat infusion broth and Luria broth). Both 1/2a and 4b strains which were grown in brain heart infusion broth for 18 and 24 hrs, showed good production of listeriolysin 'O' when evaluated by SDS-PAGE. The ammonium sulphate precipitated, dialyzed and DEAE cellulose column purified listeriolysin 'O' (56 kDa) was confirmed in the SDS-PAGE and detected in Western blotting using convalescent sera. The different fractions collected were estimated for protein concentration and was coated on to ELISA plates. An indirect ELISA was optimized using *Listeria monocytogens* PCR positive and negative sheep sera samples, different blocking buffers and anti sheep IgG conjugate . The blocking buffer containing 2% gelatin showed good results than 3% skim milk powder and 3% BSA. The hyper immune sera have been raised in two rabbits and indirect ELISA has also been performed with protein-G conjugate. Further standardization and evaluation will be carried out in due course of time.

2. Monitoring the disease burden in human risk groups

Assessment of zoonotic potential of these three diseases is the main objective of this project, samples from human were collected from risk group (veterinarians, para veterinarians, farmers/workers associated with the animals) and persons showing clinical signs.

(A) Brucellosis

Human samples were also processed in a similar way how the animal samples were processed. In addition, both IgM and IgG based ELISA were carried out (Table 30). A total of 922 samples were analyzed, of which veterinarians formed a major group.

Table 30. Human clinical samples analyzed for brucellosis during 2010-11

Risk group \ No.	Number of samples	RBPT Positive	ELISA Positive		PCR Positive
			IgM	IgG	
Veterinarians	711	67	32	139	**2***
Para veterinarians	30	-	-	2	-
Dairy farm workers	100	4	1	5	4
Piggery farm workers	39	-	-	4	-
Human showing clinical signs (Referred from hospitals)	42	-	4	4	3
Total samples analysed	**922**	**71 (7.70%)**	**37 (4.01%)**	**154 (16.70%)**	**9 (1%)**

*Only 83 samples subjected to PCR out of 711 samples.

(B) Leptospirosis

Human samples were also processed similar way as that of animal samples (Table 31). A human sample received from NIMHANS, Bengaluru, exhibiting meningitis turned out to be positive for leptospirosis. One girl child of ten months was also positive for leptospirosis diagnosed by PCR. A sample from a veterinarian wife which was suspected for brucellosis was highly positive for leptospirosis. Many of the clinical signs of brucellosis and leptospirosis overlap with many diseases and because of the proper diagnostic facility not available at many places, these two diseases in many cases go unnoticed.

Table 31. Human clinical samples analyzed for leptospirosis during 2010-11

Risk group / No.	No. of Samples subjected to isolation	Isolation positive	No. of Samples subjected to PCR	PCR Positive
Veterinarians	50	0	53	15
Para veterinarians	0	0	2	0
Dairy farm workers	0	0	22	0
Piggery farm workers	22	4	22	20
Human showing clinical signs (Referred from hospitals)	33	3	46	14
Total samples analysed	105	7	145	49

(C)Listeriosis

Six human samples (CSF) received from NIMHANS were subjected to isolation and all the samples were negative.

3. Detection of antigen in meat and their products

During 2010-11, isolation of listeria has been tried from meat and their products. A total of 48 samples comprising of four mutton, four processed frozen food, seven fresh chicken and 33 fresh fish samples were subjected to isolation of *Listeria sp*. Three isolates were obtained and they were screened by genus specific PCR and nucleotide sequencing. The sequence analysis identified the isolates as *L. innocua* (non-pathogenic).

4. Generation of molecular data

Characterization of isolates will reveal species, strain, type, susceptible hosts, pathogenicity etc., which in turn will help in understanding epidemiology and formulating control strategies.

(A) *Brucella*

The genus *Brucella* has six recognized species on the basis of host specificity. *B.melitensis* (goats and sheep), *B. abortus* (cattle and bison), *B. suis* (infecting primarily swine but also hares, rodents, and reindeer), *B. ovis* (sheep), *B. canis* (dogs), and *B. neotomae* (wood rats). At biovar level eight biovars of *B.abortus* (1-7, 9), three biovars of *B. melitensis* (1-3) and five biovars of *B. suis* (1-5) are currently recognized.

Brucella reference strains (*B. abortus* S99, *B. melitensis* 16M, *B. suis*1330 and vaccine strain *B. abortus* S19 and *B. melitensis* Rev-1) were procured from Indian Veterinary Research Institute, Izatnagar, India. Forty seven *Brucella* field isolates from animals and humans isolated earlier at PD_ADMAS were subjected to Bruce ladder PCR and AMOS (Abortus-Melitensis-Ovis-Suis) PCR. AMOS PCR has been used to identify *B. abortus* (biovar 1, 2 or 4), *B. melitensis* (all biovars), *B. ovis* and *B. suis* (biovar 1).

All the 5 reference strains and 47 field isolates showed amplification products by Bruce-ladder PCR (Fig. 62) Out of 47 field isolates 28, 14 and 5 belong to *B. abortus, B. melitensis* and *B. suis,* respectively (Table 5). In AMOS PCR, amplified product was observed in all the 28 *B. abortus* isolates and 3 out of 5 *B. suis* isolates. It was concluded that all the 28 *B.abortus* isolates belong to any one of the biovar 1, 2 or 4 and three *B. suis* isolates belong to biovar 1. The remaining two *B. suis* isolates belong to any one of the other four biovars except biovar1

(B) Leptospira

The RNA polymerase β subunit (r*poB)* gene based cloning and sequencing of leptospira samples (human-12; pig-3; horse-2) were carried out to identify or investigate the prevalence of Leptospira species / sero-group / serovar by phylogenetic analysis. The *rpoB* gene based phylogenetic analyses (Fig. 63) of pathogenic isolates showed that out of 12 human isolates, two isolates belong to *L. interrogans* Tarassovi and ten isolates to *L. inadai* sub group. Out of three pig isolates, two belong to *L. indai* sub group and one to *L. borgpetersenii* Hardjo serovar. One horse isolate belong to *L. inadai* sub group.

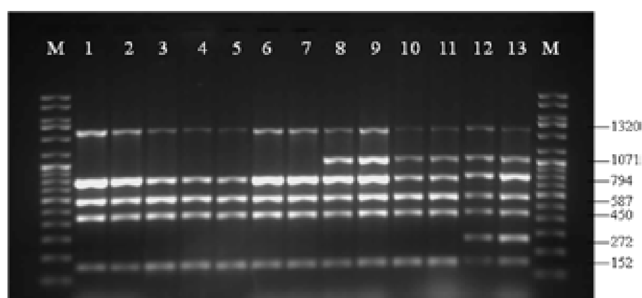


Fig. 62. Bruce ladder PCR for *Brucella* field isolates. Lanee M-100 bp ladder, Lane 1-7: *B. abortus*, Lane 8-11: *B.melitensis*, Lane 12.13: *B.suis*

(C) *Listeria*

Three isolates from fish were identified as *L.innocua* by *iap* gene specific PCR and sequencing. One isolate from buffalo serum was identified as *L.monocytogenes* by *hly* gene- specific PCR.

5. Epidemiological analysis

So far three farm investigations has been made including the human associated with those farms to assess the real zoonotic potential and the epidemiology of the diseases.

(i) A piggery farm with a herd size of 400 pigs.

(ii) A dairy farm with a herd size of 150 cows along with bulls and calves.

(iii) A stud farm having more than 25 horses.

(i) In Piggery farm

In piggery farm, after an episode of abortion storm (with 9 abortion cases) anti-*Brucella* antibodies in pig serum samples (n=40) were screened by RBPT and indirect ELISA and *Brucella* antigen by PCR assays. Out of 40 sera tested, 20, 29 and eight samples were positive in RBPT, indirect ELISA and PCR, respectively. Similarly, out of 25 human blood samples tested (22 workers, one veterinary doctor and two livestock inspectors), two, five and four were positive in RBPT, human IgG- ELISA and PCR, respectively. Simultaneously all the serum samples were inoculated in EMJH medium for the isolation of Leptospira, of which, three isolates from pigs and four from human samples, were recovered. In PCR, 23 pig and 18 human samples were positive for Leptospira specific *rpoB gene*-based PCR. From these, six human and three pig samples each were cloned and sequenced for species identification. In phylogenetic analysis of these, all the human samples and two pig samples belonged to *Leptospira inadai* species-sub group and one pig sample belonged to *L. borgpetersenii* Hardjo serovar. Interestingly, 14 out of 40 pig samples and 4 out of 25 human samples showed concurrent *Brucella* and Leptospira infection.

(ii) In Dairy farm

Brucella antibodies in cattle serum samples (n=70) were screened by RBPT and indirect ELISA while *Brucella* antigen by PCR. Out of 70 serum samples screened, 8 (11.42%) and 13 (18.57%) serum samples showed positive reaction in RBPT and ELISA respectively. Again from the 13 ELISA positives animals, serum samples were collected after 45 days and of which nine were confirmed positive for brucellosis by serology. All the samples were PCR negative for Brucella genome.

From the same farm, a total of 63 serum samples were collected from farm workers and veterinary doctors and screened for Brucella antibodies by ELISA and RBPT. Out of 63 serum samples tested, 3 (4.68%) and 7 (10.93%) were positive for *Brucella* antibody by RBPT and human IgG ELISA, respectively. All the serum samples were PCR negative. These three sero-positive individuals were advised for periodical testing.

For leptospirosis, 51 out 70 cattle samples are PCR positive. None of the human samples were found positive in PCR for leptospiral antigen. However leptospiral antibodies have to be screened in human in order to say the prevalence status of leptospira in farm workers.

iii) In stud farm

In stud farm, with a history of five abortions, serum samples (n=24) were screened by RBPT and indirect ELISA. One out of 24 serum samples was positive by RBPT and iELISA. All the plasma samples were negative for *Brucella* DNA by PCR. The aborted clinical samples (placenta, vaginal sample and fetal membranes) from 3 mares were collected and subjected to isolation using *Brucella* selective agar. *Brucella* organisms could not be isolated from the clinical samples. Similarly for leptospirosis, out of 24 horse samples, eight samples were PCR positive indicating the presence of leptospires in horses. Upon isolation, cloning and sequencing, one horse isolate belongs to *L. inadai* sub group. One horse sample which is *Brucella*

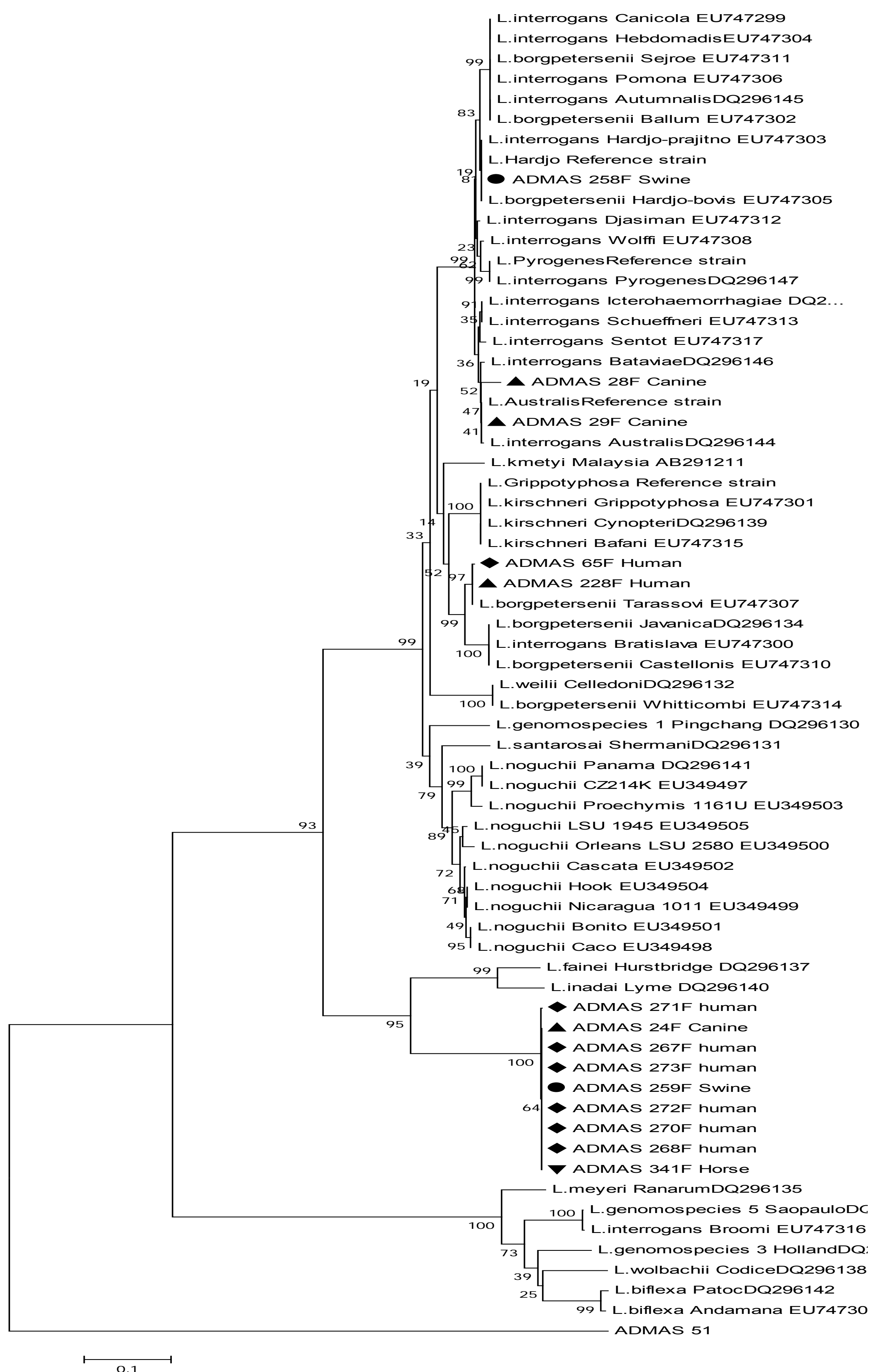


Fig. 63. Phylogenetic tree of partial nucleotide sequences of *rpoB* gene and tree topologies were evaluated by using bootstrap test in MEGA 4.1.

positive was also found leptospira positive indicating the concurrence of both leptospira and *Brucella* in the mare.

The above mentioned studies confirm the combined prevalence of brucellosis and leptospirosis in pig, cattle, horse and associated humans in the farms. In human, most of the workers had no apparent clinical signs pertaining to the diseases, even though they were positive for infections, which could precipitate at any point of time. So, there is a need for continuous monitoring of the Leptospira and *Brucella* burden in livestock and human in close proximity with each other even in the absence of specific clinical signs to combat zoonotic diseases.

Project : 18

NAIP: Bovine Mastitis: Unravelling molecular details of host-microbe interaction and development of molecular diagnostic methods

B. R. Shome, Rajeswari Shome, P. Krishnamoorthy, J. Hiremath and K. Prabhudas

Epidemiology of Mastitis: Major pathogens

Milk samples from one organized and two un-organized dairy sectors were collected and subjected to somatic cell count and electro-conductivity analysis. Further, the samples were processed for the isolation and identification of major mastitic pathogens. From 85 milk samples, a total of 205 organisms were isolated based on colony morphology and other cultural characteristics including colour, shape, size, etc and identified by genus and species specific PCR/mPCR assay standardized earlier. The most predominant pathogens were *Staphylococcus* spp. (71.70%) followed by *Streptococcus* spp. (14.15%) and *E. coli* (14.15%). The farm-wise distributions of the pathogens identified are depicted in Table 32.

Table 32. Farm-wise distribution of the mastitis pathogens isolated from bovine mastitis

Isolates	Organized farm	Unorganized Dairy Sector		Total
	Basvesh-wara Farm	Bellur	Tama-nayak-anahally	
E.coli	4	12	13	29
S.agalactiae	2	1	2	5
S.dysgalactiae	-	-	-	-
S.uberis	-	2	1	3
S. equines/bovis complex	5	3	3	11
Streptococcus urinalis	9	1	-	10
S. aureus	6	26	28	60
S. chromogenes	8	18	11	37
S. epidermidis	7	4	19	30
S. sciuri	2	4	1	7
S. haemolyticus	3	3	1	7
Staphylococcus spp.	2	4	-	6

Virulence genes

A total of 116 *S. aureus* isolates were screened for *nuc* (thermonuclease gene), *fnb*A (fibrinectin binding protein A gene), *fnb*B (fibronectin binding protein B gene), *clf*A (clumping factor A gene), *cna* (collagen binding protein), *pvl* (panton-valentine leucocidin gene) and *tst* (toxic shock syndrome toxin gene). The PCR investigation of *S. aureus* isolates showed the presence of adhesin genes at a greater percentage, *fnbA* was found in all the isolates, followed by *clfA*, *fnbB* and *cna* (Table 33). Further, a total of 173 *S. aureus* isolates were screened for a group of 12 Staphylococcus superantigens (enterotoxin and enterotoxin-like genes) namely, *Sea, Seb, Sec, See, Seg, Sei, Sel, Sen, Sem, Seo, Ser* and *Seu* (Fig. 64). Results revealed that 164 out of 173 *S. aureus* isolates showed the presence of combinations of one to eight genes whereas 9 isolates were negative for staphylococcus enterotoxin genes. The staphylococcus enterotoxin R was present in majority of isolates, 43.93% was found in combination with other genes and alone positive in 48.55% of the isolates screened. *See* was the least prevalent enterotoxin, found in 2 isolates in a combination (1.16%) (Table 34 and 35).

Table 33. Virulence gene profile of *E. coli*, *S. aureus* and *Streptococcus* species

E. coli (29 isolates)			*S. aureus* (116 isolates)			*Streptococcus* spp.(8 isolates)		
genes	positive	percentage	genes	positive	percentage	genes	positive	percentage
*stx*1A	-	-	*fnb*A	116	100	*S. agalactiae* (5 isolates)		
*stx*2	-	-	*fnb*B	72	62.07	CAMP	5	100
*cnf*1	1	3.45	*clf*A	114	98.27	*pav*A	-	-
*hly*A	-	-	*nuc*	116	100	*S. uberis* (3 isolates)		
*pap*G	-	-	*cna*	16	13.79	*skc*	3	100
*tra*T	19	65.52	*pvl*	38	32.76	*suam*	-	-
*fli*C	1	3.45	*tst*	4	3.45	CAMP	-	-

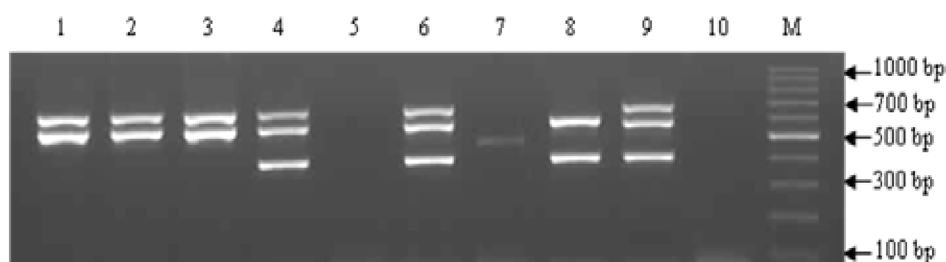


Fig. 64. Multiplex PCR for detection of Staphylococcus enterotoxin genes- *See* (484bp), *Seu* (410bp), *Sem* (687bp) and *Sei* (582bp). Lane 1-6: *S. aureus* field isolates, Lane 7: ATCC 25904 *S. aureus*, Lane 8: ATCC 43300 *S. aureus*, Lane 9: ATCC 49775 *S. aureus*, Lane 10: No template control; Lane M: 100 bp DNA marker.

Table 34. Staphylococcal superantigens (enterotoxin and enterotoxin like genes) of *S. aureus* isolates obtained from cases of bovine mastitis.

Sl. No	Target gene	Oligonucleotide primer	Reference	Amplicon Size	Anneal. temp	No. of isolates positive	Percentage (173 isolates)
1	*Se*A	GGTTATCAATGTGCGGGTGG CGGCACTTTTTTCTCTTCGG	Mehrotra *et al* 2000	102 bp	57 ºC	46	26.58
2	*Se*B	GTATGATGATAATCATGTATCAGCAATA CGTAAGATAAACTTCAATCTTCACATC	Patrick *et al*., 2007	625 bp	48 ºC	3	1.73
3	*Se*C	CTTGTATGTATGGAGGAATAACAA TGCAGGCATCATATCATACCA	Monday and Bohach 1999	283 bp	57 ºC	7	4.05
4	*Se*E	GGTAGCGAGAAAAGCGAAG GCCTTGCCTGAAGATCTAGCTC	Patrick *et al*., 2007	484 bp	57 ºC	2	1.16
5	*Se*G	TGAATGCTCAACCCGATCCTAAAT CAAACCAAAAACTTGTATTGTTCTTTTCA	Patrick *et al*., 2007	588 bp	57 ºC	31	17.92
6	*Se*I	CGTATGCTCAAGGTGATATTGGTG AAAAACTTACAGGCAGTCCATCTCC	Patrick *et al*., 2007	582 bp	57 ºC	8	4.62
7	*Se*L	CACCAGAATCACACCGCTTA TCCCCTTATCAAAACCGCTAT	Patrick *et al*., 2007	411 bp	57 ºC	34	19.65
8	*Se*M	TTTTGCTATTCGCAAAATCATATCGCA TCAACTTTCGTCCTTATAAGATATTTCTAC	Patrick *et al*., 2007	687 bp	57 ºC	8	4.62
9	*Se*N	CGTGGCAATTAGACGAGTCA GCATCCTTTTTGTATGTTACCG	Present Study	440 bp	57 ºC	55	31.79
10	*Se*O	TAGTGTAAACAATGCATATGCAAATG ATTATGTAAATAAATAAACATCAATATGATA	Patrick *et al*., 2007	722 bp	48 ºC	8	4.62
11	*Se*R	TTCAGTAAGTGCTAAACCAGATCC CTGTGGAGTGCATTGTAACGCC	Hwang *et al* 2007	368 bp	48 ºC	160	92.49
12	*Se*U	ATGGCTCTAAAATTGATGGTTCTA GCCAGACTCATAAGGCGAACTA GGTGATTATTCATGGTTAAG	Patrick *et al*., 2007	410 bp	57 ºC	-	-

Similarly, five isolates of *S. agalactiae* were screened for *cfb* (CAMP factor) and *pav*A (fibronectin binding protein gene) and three isolates of *S. uberis* were screened for *skc* (streptokinase gene), CAMP factor gene and *sua* (*S. uberis* adhesion molecule gene). All the isolates of *S. agalactiae* were found to possess *cfb* genes (Table 33). The *S. uberis* isolates were found to possess plasminogen activator gene, *skc* in all the isolates. Twenty nine isolates of *E. coli* were screened for virulence genes, namely *tra*T (serum resistance protein gene) *fli*C (flagellin protein gene), *stx*1 (shiga toxin 1 gene), *stx*2 (shiga toxin 2 gene), *hly*A (enterohaemolysin A gene), *pap*G (pfimbrial adhesions gene), *cnf*1 (cytotoxic necrotizing factor1gene). Majority of the *E. coli* isolates (65.52%) were found to possess *tra*T gene (Table 33).

Table 35. Distribution of Staphylococcus enterotoxin genes of bovine *S. aureus* isolates across the farm

SEs	VC (8)	AK (9)	CH (28)	HF (68)	T (28)	B (26)	BF (6)	No. of isolates (%)
M+I+R+O+N+L+G+A	-	2	-	-	-	-	-	2 (1.16)
M+I+R+O+N+L+G	-	1	-	-	-	-	-	1 (0.58)
M+I+R+O+N+G+A	-	1	-	-	-	-	-	1 (0.58)
M+I+R+O+N+A	-	2	-	-	-	-	-	2 (1.16)
R+N+L+G+C+A	6	-	-	-	-	-	-	6 (3.47)
M+I+R+O+N+G	-	2	-	-	-	-	-	2 (1.16)
E+R+N+L+G+A	-	1	1	-	-	-	-	2 (1.16)
R+N+L+C+A	1	-	-	-	-	-	-	1 (0.58)
R+N+L+G+A	-	-	2	1	-	-	-	3 (1.73)
R+N+L+A	1	-	2	3	-	-	-	6 (3.47)
R+N+G+A	-	-	3	-	-	-	-	3 (1.73)
R+N+G	-	-	2	3	3	-	-	8 (4.62)
R+N+A	-	-	3	-	-	-	-	3 (1.73)
R+N+L	-	-	2	3	-	-	-	5 (2.89)
R+G+A	-	-	1	-	-	-	-	1 (0.58)
R+B+G	-	-	-	1	-	-	-	1 (0.58)
R+B+N	-	-	-	1	-	-	-	1 (0.58)
R+L+A	-	-	-	3	-	-	-	3 (1.73)
R+N	-	-	5	3	1	-	-	9 (5.2)
R+G	-	-	1	-	-	-	-	1 (0.58)
R+A	-	-	-	8	-	2	-	10 (5.78)
R+L	-	-	1	4	-	-	-	5 (2.89)
R	-	-	5	35	22	20	2	**84 (48.55)**
A	-	-	-	1	-	-	2	3 (1.73)
B	-	-	-	-	-	1	-	1 (0.58)
Negative	-	-	-	2	2	3	2	9 (5.2)
Total no. of isolates								**173**

Antibiotic resistance of *Staphylococcus* spp.

The emergence of antibiotic resistant strains of bacteria especially methicillin resistance of Staphylococci is an alarming problem in the health sectors worldwide. We investigated the antibiotic resistance of 440 isolates of staphylococci (267 CNS and 173 *S. aureus*) by conventional disc diffusion method and found that the isolates were resistant to the commonly used antibiotics like Ampicillin (30.5%), Amoxyclav (14.1%), Penicillin (13.9%), Cloxacillin (5.71%), Gentamicin (3.43%), Tetracyclin (2.53%), Vancomycin (1.57%), Cephalothin (0.57%) and Erythromycin (0.4%) and sensitive to Oxacillin and Cephalothin. Interestingly, all the *S. aureus* were methicillin sensitive while seven isolates of CNS were resistant to methicillin by disc diffusion.

To detect the genetic resistance of Staphylococcus to methicillin, *mec*A, the most important gene carried on the Staphylococcal cassette responsible for methicillin-resistance was targeted and was amplified using the published primers (Hassanian Al-Talib *et al* 2009). Also to differentiate MRSA from MSSA and R-CNS (Methicillin resistant CNS), a mPCR was developed targeting *fem* A/B genes in addition to *mec*A (Table 36, Fig. 65), these genes have been found to encode proteins which considerably affect the level of methicillin resistance of *S.aureus*. All the 173 isolates of *S. aureus* were subjected for the mPCR assay and it was found that all the isolates were *mec*A negative.

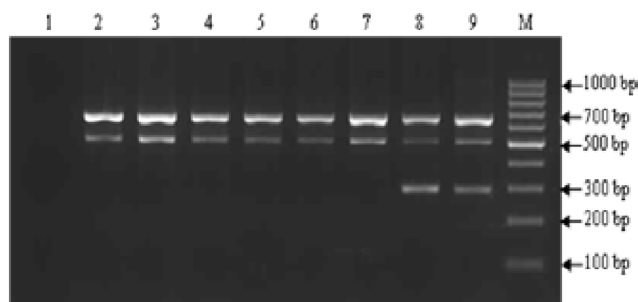


Fig. 65. mPCR targeting *mec*A (293bp), *fem*A (509bp) and *fem*B (651bp) genes for detection of methicillin resistance in *S. aureus*: Lane 1- No template control, Lane 2 to 7- *S. aureus* field isolates, Lane 8,9- ATCC 43300 *S. aureus*, Lane M- 100bp DNA marker.

To detect the methicillin resistance of CNS, all the 349 isolates were screened for the presence of *mec*A gene. Twelve isolates comprising *S. haemolyticus* (7), *S. epidermidis* (3) and *S. chromogenes* (1) and *Staphylococcus* spp. (1) were found to carry *mec*A. Interestingly, of the 12 *mec*A positive CNS isolates, only three isolates showed phenotypic expression of resistance, four isolates showed intermediary resistance and five isolates were sensitive. Further, three isolates showed phenotypic resistance irrespective of the presence of *mec*A. The *mec*A positive field isolates and ATCC 43300 *S. aureus* (Reference strain) were subjected for Staphylococcal Chromosomal Cassette (SCC) typing. SCC is made up of two main genetic elements: the *ccr* gene complex and the *mec* gene complex. The *ccr* gene complex includes three recombinase genes, *ccr*A, *ccr*B, and *ccr*C, responsible for the mobility of the cassette. The *mec* gene complex (class A-D) is made up of multiple regulatory genes responsible for the expression of genes carried on the cassette. To date, there are eight distinct SCC*mec*- types in *S. aureus* based on the different combinations of the *ccr* gene complex and the *mec* gene complex. Primers for amplification of *ccr* gene and *mec* gene complex (table 36) were adapted from Kondo *et al.* (2007). SCC typing of the isolates revealed that all the field isolates were of class C *mec* and *ccr* type C, whereas ATCC *S. aureus* was of class A *mec* type and *ccr* complex was untypable (Fig. 66) Further sub-typing of the *mec* class C as C1 and C2 was not possible with the published primers to determine if the isolates belong to SCC- *mec* class V or VII. Results indicated that the isolates belonged to neither class V nor class VII, hence further efforts need to be directed to sequence the entire SCC-*mec* element of around 30Kb to determine the new type.

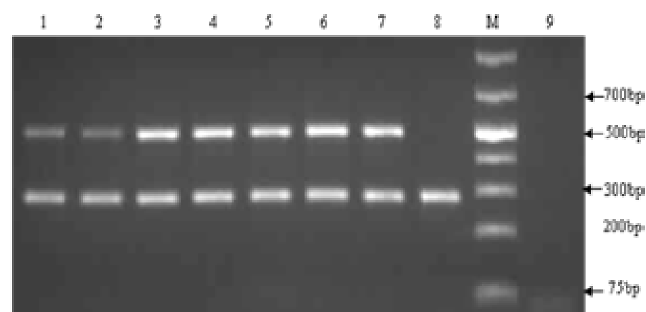


Fig. 66. mPCR for amplification of *ccr* gene complex type with *mec*A showing the amplification of *ccr*C gene (518bp) and *mec*A gene (286bp). Lane 1 to 6 – Methicillin resistant S. haemolyticus, Lane 7: Methicillin resistant S. chromogenes, Lane 8- Methicillin resistant ATCC 43300 S. aureus, Lane M- 100bp DNA marker and Lane 9- No template control.

Recent DNA sequence analysis of the *S. aureus* USA300-0114 genome identified a novel genetic region, designated the arginine catabolic mobile element (ACME), that potentially enhances virulence and the ability to colonize humans. In methicillin-resistant staphylococci, ACME is always adjacent to SCC*mec*

Table 36. Details of primers targeting genes attributing to antibiotics resistance of Staphylococcus

Sl. No	Target gene	Oligonucleotide primer	Reference	Amplicon size	Annealing temp
1	*fem*AF	AGACAAATAGGAGTAATGAT	kobayashi *et al.*, 1994	509 bp	56 ºC
	*fem*AR	AAATCTAACACTGAGTGATA			
2	*fem*BF	TTACAGAGTTAACTGTTACC	Trujillo *et al.*, 2003	651 bp	56 ºC
	*fem*BR	ATACAAATCCAGCACGCTCT			
3	*mec*A1F	ACGAGTAGATGCTCAATATAA	Hassanain Al-Talib *et al.*, 2009	293 bp	56 ºC
	*mec*A1R	CTTAGTTCTTTAGCGATTGC			
4	*mec*A2 F	TGCTATCCACCCTCAAACAGG	Kondo *et al.*, 2007	286 bp	57 ºC
	*mec*A2 R	AACGTTGTAACCACCCCAAGA			
5	*ccr*A1 R	AACCTATATCATCAATCAGTACGT	”	695 bp	57 ºC
	*ccr*A2 R	TAAAGGCATCAATGCACAAACACT			
6	*ccr*A3 R	AGCTCAAAAGCAAGCAATAGAAT	”	1791 bp	57 ºC
	*ccr*B F	ATTGCCTTGATAATAGCCTTCT			
7	*ccr*A4 F	GTATCAATGCACCAGAACTT	”	1287 bp	57 ºC
	*ccr*B4 R	TTGCGACTCTCTTGGCGTTT			
8	*ccr*C F	CCTTTATAGACTGGATTATTCAAAATAT	”	518 bp	57 ºC
	*ccr*C R	CGTCTATTACAAGATGTTAAGGATAAT			
9	Class A *mec*	CATAACTTCCCATTCTGCAGATG	”	1963 bp	57 ºC
	mA7	ATATACCAAACCCGACAACTACA			
10	Class B *mec*	ATGCTTAATGATAGCATCCGAATG	”	2827 bp	57 ºC
	mA7	ATATACCAAACCCGACAACTACA			
11	Class C *mec*	TGAGGTTATTCAGATATTTCGATGT	”	804 bp	57 ºC
	mA7	ATATACCAAACCCGACAACTACA			
12	ACME *arc*A F	CTAACACTGAACCCCAATG	Diep *et al.*, 2008	1946 bp	-
	ACME *arc* A R	GAGCCAGAAGTACGCGAG			
13	*Opp*3A F	GCAAATCTGTAAATGGTCTGTTC	Diep *et al.*, 2008	1183 bp	-
	*Opp*3A R	GAAGATTGGCAGCACAAAGTG			
14	N *arc*A F	CGTCCACGAACAAGT	Pi *et al.*, 2009	736 bp	52 ºC
	N *arc*A R	GACCCAATGCCTAAC			

elements and its integration and excision is believed to be catalysed by the SCC*mec* recombinase encoded by the *ccr* gene. ACME contains two gene clusters *arc* (encoding a secondary arginine deiminase system) and *opp*-3 (encoding an ABC transporter). The *mec*A positive field isolates and ATCC 43300 *S. aureus* were screened for the presence of ACME using PCR based assay targeting both the gene clusters (table 4) and the results revealed that neither the *mec*A positive field isolates nor the ATCC strain of *S. aureus* possessed the *arc* and *opp*-3 genes.

DNA fingerprinting by pulsed-field gel electrophoresis (PFGE) is considered to be one of the most reliable, discriminatory, and reproducible typing procedures to allow the detection of high degree DNA polymorphism. PFGE analysis of the *mec*A positive CNS isolates was done as previously described by Arakere *et. al.*(2004). The preliminary PFGE analysis of representative *mec*A positive isolates showed

distinct species-wise banding patterns indicating that the isolates within the species were homogenous (Fig. 67)

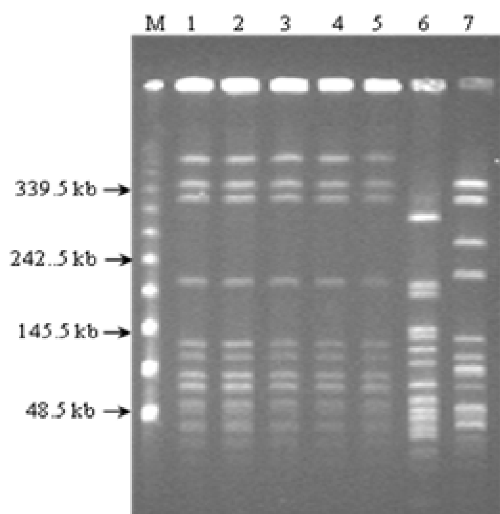


Fig. 67. Representative methicillin resistant Staphylococcal pulse field gel electrophoretic pattern of DNA digested by *sma*I. Lane M: Lambda ladder PFGE marker; Lane 1-5: Methicillin resistant *S. haemolyticus*, Lane 6: Methicillin resistant *S. chromogenes*; Lane 7: Methicillin resistant *S. epidermidis*.

Molecular epidemiology of mastitis pathogens

S. aureus isolates does not belong to ST 398

Several studies have reported the frequent transmission of Sequence type 398 among humans from animals like horse, pig and cows. Hence, the *S. aureus* isolates from bovine mastitis were screened to ascertain if they belonged to ST 398 using a set of specific primers set 1F- 5'GATCCCAGAATACTTAA ATA3' and 1R- 5' TGACCGTAA TCTTGTAAATA3' (197 bp) and 2F- 5'CATTCATCACAC GTATATTC3' and 2R-5'GGTGATTA TTCATGGTTAAG3'(140 bp) designed by Wamel *et al.* (2010). PCR results revealed that none of the *S. aureus* isolates belonged to ST 398.

Typing of *S. aureus* based on Staphylococcal protein A gene polymorphism

The staphylococcal protein A (*spa*) typing was used to study the genetic diversity of the *S. aureus* isolates of bovine mastitis. The *spa* gene possesses a variable number of nucleotide repeats of 21-27bp, the most common being 24bp repeats. A total of 173 *S. aureus* isolates were subjected to *spa* typing (Fig.5) using the designed primers *spa* F- 5' ACGTAACGGCTTCATCCA3' and *spa* R- 5' TCCACCAAATACAGTTGTACCG3', to yield an amplicon of 589 bp. The individual isolates were amplified with *spa* specific primer to yield varying size of amplicon from 373 bp to 589 bp, which were sequenced. The sequences were edited with signature sequences to determine the number of 24bp repeats and repeat successions to obtain the *spa* type using the RIDOM *spa* server. The analysis of isolates revealed 26 *spa* types of which 11 were new *spa* types and one remained unidentified *spa* type (Table 37). The 11 new types were submitted to RIDOM database with the following designations: t7286, t7287, t7288, t7680, t7681, t7682, t7683, t7684 t7695, t7696 and t7867. The three major types namely t267, t359 and t6877 were predominant among the farms. The *spa* type t267 has a set of 10 repeats (r07r23r12r21r17 r34r34r34r33r34) and in t359 which has 9 repeats (r07r23r12r21r17r34r34r33r34) a deletion of repeat r34 was observed at 8th position which is suggestive of the possibility of some evolutionary relationships between the two *spa* types. The *spa* type t6877 was similar to that of t267 except for a single nucleotide variation at 10th repeat (nt 20: C to A). The *spa* type t267 and t359 have been reported worldwide from cases of bovine mastitis, whereas the association of t6877 with bovine mastitis has been reported for the first time through our study.

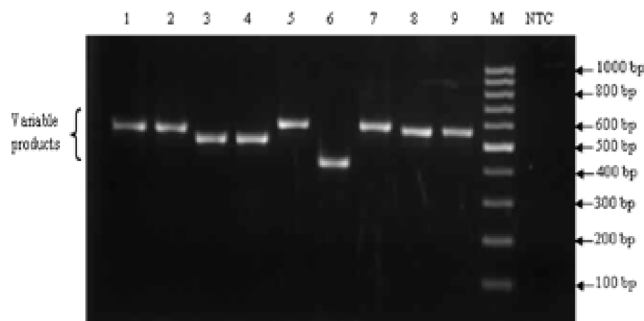


Fig. 68. Amplified product of *spa* gene *S. aureus* isolates. Lane 1-9: *S. aureus* field isolates (Variable amplicon of 421 bp to 589 bp). M: 100 bp marker and NTC: No template control.

Quantitative multiplex PCR for detection of 14 mastitis pathogens

With the aim to facilitate automation of the laboratory work flow in surveillance and monitoring of mastitis diagnosis, the work on Real time PCR to quantify the specific microbial load in milk samples has been undertaken. Based on the prevalence studies and reports on mastitis, the assay has been designed to target the bacteria that are most relevant and frequently associated with sub-clinical and clinical mastitis. Primers and probes were designed (TIB MOLBIOL

Table 37. Genotyping/*spa* typing of the *S. aureus* isolates collected from bovine mastitis and their distribution.

Sl. No	*spa* type	Repeats	No. of repeats	Farms*							No. of Isolates (173)	% of strains
				KVA FSU	AK	HF	CH	T	B	BF		
1	t267	r07r23r12r21r17r34r34r34r33r34	10	-	-	34	7	2	1	-	44	25.43
2	t359	r07r23r12r21r17r34r34r33r34	9	3	-	3	11	13	11	1	42	24.27
3	t6877	r07r23r12r21r17r34r34r34r33r204	10	-	-	29	-	-	-	-	29	16.76
4	**t7696**	r07r23r12r437r17r34r34r34r33r34	10	-	-	-	-	-	9	-	9	5.20
5	**t7286**	r07r16r12r23r2r34r34	7	-	7	-	-	-	-	-	7	4.05
6	t3992	r26r23r12r21r17r34r34r33r34	9	5	-	-	-	-	-	-	5	2.89
7	t2770	r07r23r12r12r21r17r34r34r33	10	-	-	-	2	2	-	1	5	2.89
8	t311	r26r23r17r34r20r17r12r17r16	9	-	-	-	-	4	-	-	4	2.31
9	**t7287**	r125r21r17r34r12r23r2r12r23	9	-	-	-	3	-	-	-	3	1.73
10	**t7680**	r07r438r12r437r17r34r34r34r33r34	10	-	-	-	-	2	-	1	3	1.73
11	t2478	r26r23r21r17r34r34r16r34r33r13	10	-	-	2	-	-	-	-	2	1.16
12	t6861	r07r16r21r17r34r34r33r34	8	-	-	-	-	-	2	-	2	1.16
13	t008	r11r19r12r21r17r34r24r34r22r25	10	-	-	-	-	-		2	2	1.16
14	t2802	r07r23r21r17r34r34r34r33r34	9	-	-	-	1	-	-	-	1	0.58
15	t1200	r07r23r34	3	-	-	-	1	-	-	-	1	0.58
16	t2104	r11r19r12r34r24r34r22r25	8	-	1	-	-	-	-	-	1	0.58
17	t2915	r11r25r25	3	-	-	-	1	-	-	-	1	0.58
18	t5109	r07r23r17r34r34r34r33r34	8	-	-	-	1	-	-	-	1	0.58
19	**t7288**	r125r12r34r12r23r02r12r23	8	-	-	-	1	-	-	-	1	0.58
20	**t7681**	r26r23r12r34r02r17r12r12r17r16	10	-	-	-	-	1	-	-	1	0.58
21	**t7682**	r125r12r23r02r12r23	6	-	-	-	-	1	-	-	1	0.58
22	**t7683**	r07r23r12r21r149r34r34r33r34	9	-	-	-	-	1	-	-	1	0.58
23	**t7684**	r07r23r21r34r34r33r34	7	-	-	-	-	-	1	-	1	0.58
24	**t7695**	r07r23r21r34r34r34r33r34	8	-	-	-	-	-	1	-	1	0.58
25	**t7867**	r16r12r23r02r02r34r34	7	-	1	-	-	-	-	-	1	0.58
26	unidenti-fied	**Unnamed**	1	-	-	-	-	2	1	1	4	2.31

*KVAFSU: Karnataka Veterinary Animal Fisheries Sciences University Farm; AK: Ashok Kumar Farm, Doddaballapur; HF: Hessarghatta Govt Farm; CH: Chikkanayakanahalli; T: Tamanayakanahally; B: Bellur; BF: Basveshwara Farm

Synthese labor GmbH, Berlin) for 14 important mastitis pathogens viz. *E. coli, S. agalactiae, S. dysgalactiae, S. aureus, S. chromogenes, S. sciuri, S. simulans, S. uberis, S. epidermidis, S. haemolyticus, Corynebacterium bovis, Mycoplasma bovis, Mycoplasma agalactiae* and *S. bovis*. For convenience, the assays protocol has been designed to split the 14 targets into 3 separate multiplex real time PCR reactions. The specificity of the primers and probes were ensured *in silico* by BLAST analysis. The first set of primer - probe (S1) comprising of 5 organisms (*E. coli, S. agalactiae, S. aureus, S. chromogenes* and *S. dysgalactiae*) has been synthesized (TIBMOL, Germany through M/S Roche Diagnostics India Pvt. Ltd, Chennai) and the other two sets (S2 and S3) are in the process of synthesis. *E. coli* and *S. agalactiae* specific probes were synthesized with the reporter dye 6-carboxyfluorescein (FAM) covalently linked to the 5'end. The characteristics of the primers and the probes used in this study are summarized in Table 6.

In order to find out the most efficient pair for a TaqMan PCR, for each organism four different primers were synthesized, to be tested in an initial run together with the probe in all four possible combinations. Individual Conventional PCR followed by real time PCR for the first set (S1) of Primer probe has been optimized. The primer- probe has been found to work best at Tm 60°C. Currently multiplex Real time PCR reaction is under progress to optimize the multiplex conditions for Set 1.

*pho*A- Alkaline phosphatise gene, *gro*EL- Molecular chaperone, *nuc*- Thermonuclease gene, *sod*A- Superoxide dismutase gene

Table 38. Details of the primer- probe (S1) designed and synthesised for quantitative multiplex PCR

Organism	Gene	Primers and probes	
E. coli	*pho*A	F	gTCCgggTAACgCTCTggA
		S	ggAAAAACgCTgCgTgAAC
		A	CTTCCgTCACCgAATTCAgTg
		R	CCAgCgCACTggCATATTg
		Probe	**FAM-TCgCTCACCAACTgATAACCACgC-BBQ**
S. agalactiae	*gro*EL	F	ACAgCTACgACATTgAAAgTTCCAC
		S	AgAACAAgCgTTgggAgAgC
		A	AgAggTTCTTAAAACAAATCgTCCg
		R	ACgAgAAAATggTCTCTgAACTTgAg
		Probe	**FAM-CTCCATCAACATCATCAgCgATgATTAgCA-BBQ**
S. aureus	*nuc*	F	AAATTACATAAAgAACCTgACA
		R	gAATgTCATTggTTgACCTTTgTA
		Probe	**LC 610-AATTTTAACCgTATCACCATCAATCgCTTT- BBQ**
S. chromogenes	*sod*A	F	gCgTACCAgAAgAAgATAAACAAACTCCT
		S	gCggTCACTTAAACCACTCATTATT
		A	gCTTTgTCTgCAAATTCTTTTTTgA
		R	AAAACgAgCTgCTgCTTTgTC
		Probe	**LC640-AAAACgAgCTgCTgCTgCTTTgTC- BBQ**
S. dysgalactiae	*gro*EL	F	ACCAAATCCTggAgCTTTgACA
		S	CTTTgACAgCAACCACATTgAAA
		A	CCCATTgCTTgAggAAgTTCTTAA
		R	CCAAgATATTCTCCCATTgCTTgAg
		Probe	**LC670-CCACgAATTTTATTCAAAACAAgggTTggA-BBQ**

Experimental intramammary infection in mice

A Swiss-albino murine model was established (Figs. 69 and 70) to study the changes that occur upon intramammary infections at cellular and molecular level. Seven prototype strains of Staphylococci (3), Streptococci (3) and *E. coli* (1) were selected based upon their genotypes and virulence potential for experimental induction of IMI; of these, the experimental analysis of one strain each of *S. aureus* and *S. agalactiae* have been completed (Table 39).

The amount of bacteria to be inoculated into the mice mammary gland for infection studies varies depending upon the bacterial species. Different doses of the bacterial inoculum have been used in the experimental IMI by various researchers. For *E. coli* intramammary infections, inoculation dose ranging from 10-20cfu/50 µl to 10^6-10^7cfu/ml, in case of *S. agalactiae*, 10^7-10^8cfu/ teat and for *S. aureus*, 1.4 – 3.0 x10^2cfu/100 µl to 10^5-10^6cfu/50 µl were used. As the inoculation dose remained inconclusive, a pilot study was undertaken to arrive at a desired dose of inoculum for IMI of each bacterial species. The dose standardized for intramammary inoculations were 5x10^3cfu/50µl for *S. aureus* and *S. agalactiae* and 3x10^3cfu/50µl for *E. coli*. The infected mammary tissues were collected in time course manner (2, 4, 8, 12, 24, 48 hrs post innoculation) and used for analysis of cellular changes by histopathological studies and for evaluation of inflammatory cytokines expression at RNA transcript level.

Table 39. Details of the prototype strain of bacteria selected for experimental intramammary infection

Organism	Characteristics
S. aureus (HF 14LY)	*spa* type t6877, possess *nuc*, *fnb*A, *fnb*B, *clf*A, *cna*, *pvl* and *tst*
S. agalactiae (VC 394)	ST-483 (unique), possess *pav*A gene

Fig. 69. Individual ventilated cages used for experimental intramammary infection in swiss albino mice.

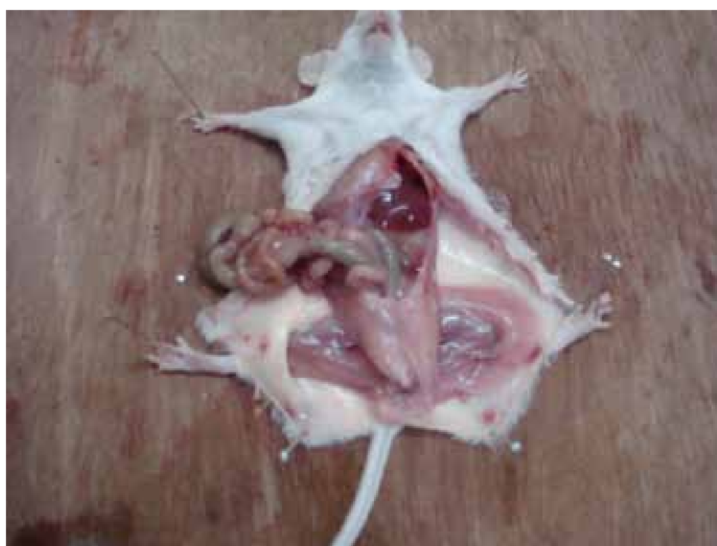


Fig. 70. Dissected mice showing the changes in mammary gland tissue after 8 hours of intra mammary infection

Histopathological studies for evaluation of cellular changes in mammary tissue in response to *S. aureus* and *Streptococcus sp.* in time course assay

Staphylococcus aureus (*spa* type 6877):

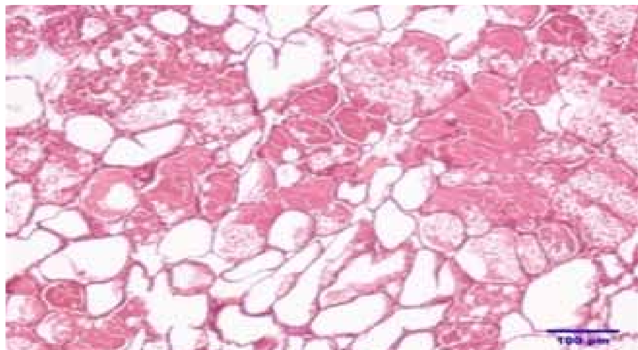


Fig. 71a. PBS Control – The alveoli are lined by single layer of cuboidal epithelial cells with secretory acini and normal interlobular septa. Alveolar lumen filled with milk secretion containing fat globules and protein. Deep pink staining was observed in the lumen due to presence of proteinaceous material. The alveoli were surrounded by few macrophages in the interlobular space which is normal. 2 and 4 hours – The mammary gland sections were similar to control mammary gland sections (H & E).

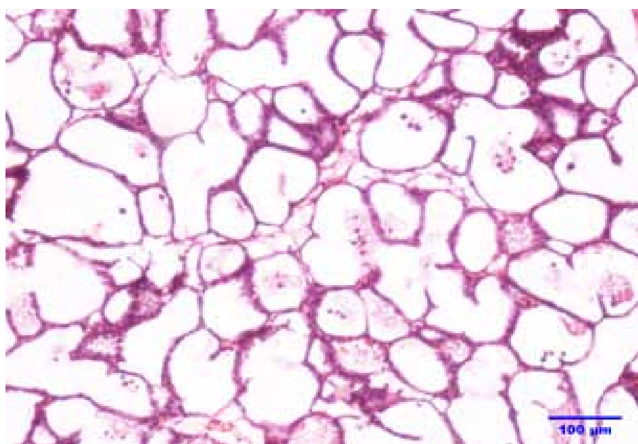


Fig. 71b. 8 hours – Alveolar epithelial cells were discontinuous, ruptured and fused with adjacent alveoli to form large alveoli. Mild infiltration of alveolar macrophages and neutrophils in the alveolar lumen from the interstitial space was observed (H & E).

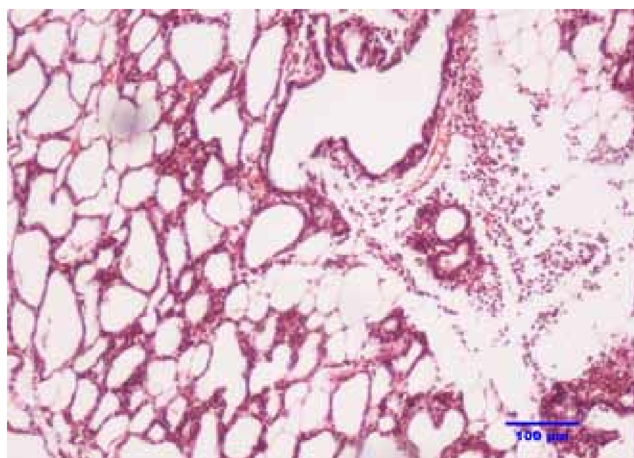


Fig. 71c. 12 hours – Moderate loss of alveolar epithelium and rupture of interlobular septa was observed. Moderate polymorpho-nuclear and mononuclear cell infiltration was noticed in the interlobular space (H & E).

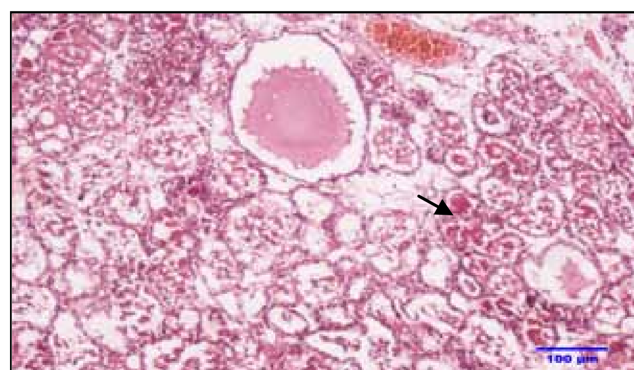


Fig. 71d. 24 hours - Alveolar epithelium showing loss of normal architecture. Necrotic debris mixed with large number of mononuclear cells was seen in the acinar lumen .Severe infiltration of mononuclear cells in the interlobular space along with marked congestion of blood vessels was also seen (H & E).

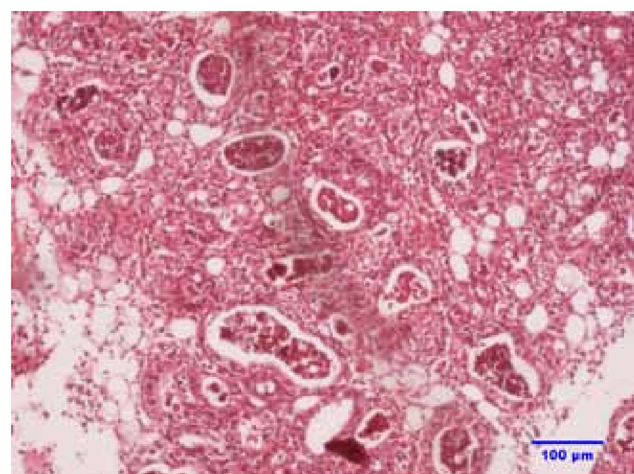


Fig. 71e. 48 hours – Severe necrosis of alveolar epithelium along with infiltration of mononuclear cells and necrotic debris was observed in the duct. Interlobular space filled with numerous mononuclear inflammatory cells and congestion of blood vessels was also observed (H & E).

The average mammary gland weight and percentage body weight of mammary gland was plotted against the various time intervals in a graph (Fig. 72). In case of *S. aureus* (t6877) induced IMI, there was initial increase in the weight of mammary gland at 8hrs after inoculation followed by a steady decrease thereafter. The initial increase in weight may be attributed to inflammatory changes during the period of 8 hours of inoculation.

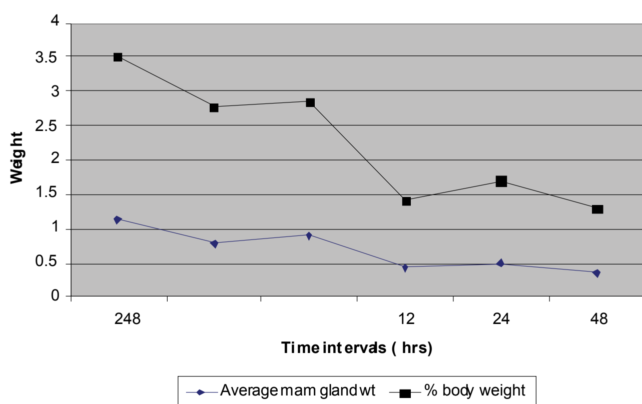


Fig. 72. Mammary gland weight during different time intervals after inoculation of *Staphylococcus aureus* (HF 14LY)

S. agalactiae (ST-483):

PBS Control –Alveolar lumen filled with milk secretion containing fat globules and protein. Deep pink staining was observed in the lumen due to presence of proteinaceous material. The alveoli were surrounded by few macrophages in the interlobular space which is normal. **2 and 4 hours** – The mammary gland sections were similar to control mammary gland sections

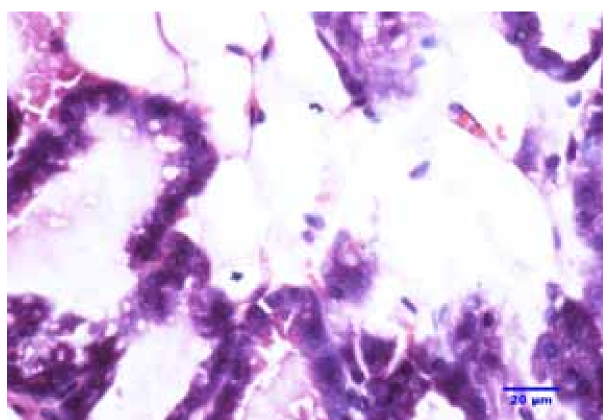


Fig. 73a. 8 hours – Alveolar epithelial cells showed mild degeneration along with mild infiltration of neutrophil and macrophages in the lumen (H & E).

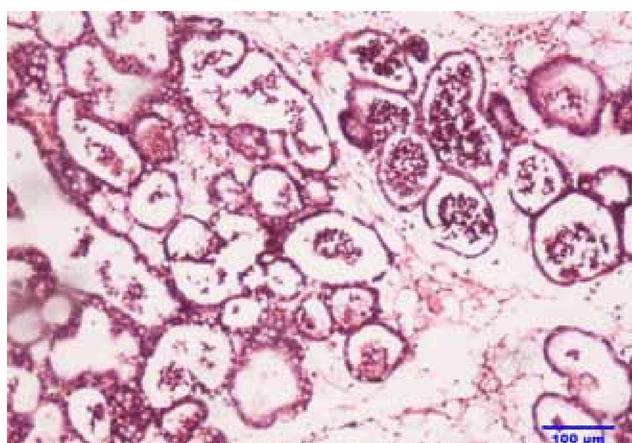


Fig. 73b. 12 hours - Alveolar epithelium showed rupture and fusion of adjoining acini at few places. Alveolar lumen and interlobular space showed moderate infiltration of neutrophils and mononuclear cells (H & E).

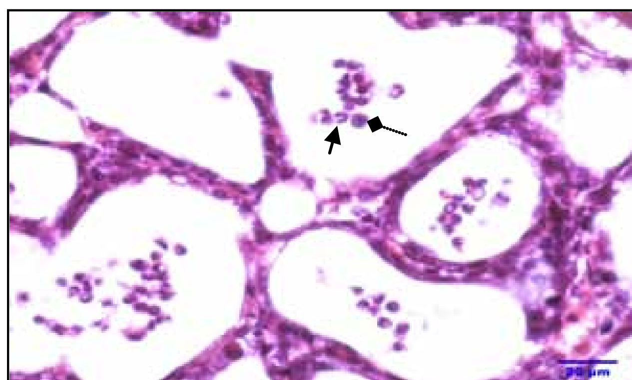


Fig. 73c. 24 hours – Alveolar lumen showing moderate infiltration of neutrophils (triangular arrow head) and macrophages (square arrow head) with congestion of blood vessels (H & E).

In conclusion, severe necrosis of alveolar lumen and numerous infiltrations of inflammatory cells were observed in mammary tissue infected with *S. aureus* (t6877). These inflammatory changes started early at 8 hours post-infection which persistently enhanced until 48 hours. However, *S. agalactiae* (ST483) inoculated mammary gland showed a moderate level of cellular infiltration at 12 hours without necrosis of alveolar epithelium. Based on the above observations, it can be concluded that *S. aureus* (t6877) induced more severe cellular damage to mammary gland when compared to *S. agalactiae* (ST483).

Evaluation of RNA transcript level in tissue for selected inflammatory mediators in response to *S. aureus* and *S. agalactiae* in time course assay

Knowledge about the molecular nature and time point of activation of the switches dictating the nature

of inflammation (as clinical or subclinical) as an outcome of infection is crucial for understanding of the pathogen specific reaction of the host and might be helpful for designing preventive measures to reduce the incidence of mastitis.

Many different mediators of inflammation are expressed at different time point after an exposure to the pathogen or stimulus. Pro-inflammatory cytokines promote inflammation quickly after the recognition of pathogen and anti-inflammatory cytokines suppress and confine the activity of pro-inflammatory cytokines. Expression profile of genes associated with immune response mechanisms in mastitis induced by *S. aureus* (*spa* t6877) and *S. agalactiae* (ST-483) was done by Real Time PCR aasay using Universal Probe Library for relative quantification of the expression of genes: IL-2, IL-4, IL-6, IL-12, GMCSF, IFNγ, TNF-α, TLR-2, TLR-4, TLR-9, TLR-11,TLR-12, CD14, IL1β, RANTES, Lactoferrins, CxCl1, CxCl5, C3, SAA3, TLR-13. This study demonstrates a pathogen related response in mRNA expression of pathogen recognition receptors (mainly TLRs), pro-inflammatory cytokines, and chemokines in the infected mammary gland.

Staphylococcus aureus (*spa* t6877):

Mouse mammary gland challenged with *S. aureus* (t6877) showed maximal increase in the expression of IL6, IL12, TNF-α, IFN-γ, TLR9, IL1b, RANTES, CXCl1 and CXCl5 at 24th hour post infection (11-54 folds). No significant changes were observed in expression of TLR2 and TLR 4, while TLR 9 showed remarkable increase of 10 fold at 24th hour, emphasizing its function in recognizing gram-positive bacteria. IL12 was up regulated (30.86 fold) at 2 hours post challenge attaining a peak (54.35 fold) at 24th hour. All the inflammatory mediators showed a sharp decline at 48th hour, except CD14 which showed maximum expression (39.32 fold) at this hour (Fig. 74). This short lived inflammatory response after *S. aureus* infection as evidenced by sharp decline in the expression of the genes at 48th hour post infection could favour the establishment of *S. aureus* in the mammary gland.

S. agalactiae **(ST-483)**

S. agalactiae is a contagious mastitis-causing pathogen highly adapted to survive in the bovine mammary gland. *S. agalactiae* induced infection revealed significant up regulation of 7 genes, of which, four genes *viz.* GMCSF, CD14, C3 and SAA3 peaked at

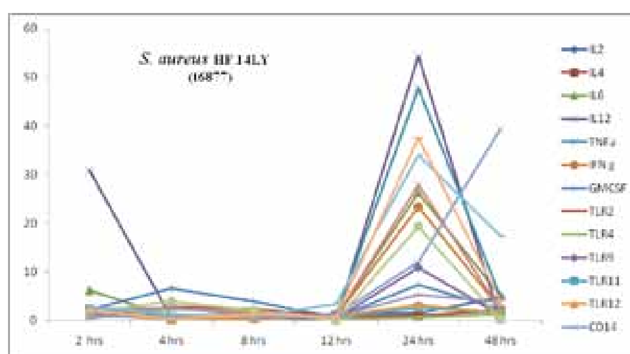


Fig. 74. Alteration in the mRNA concentrations of 18 immuno regulatory genes in mammary tissue after challenge with *S. aureus* (*spa* type t6877) strain in time course manner. Shown are values for mean fold induction at times after challenge relative to the mRNA concentration measured for unstimulated cells.

the very initial course of disease *i e.*, 2 hours followed by a sharp decline at 4th hour post innoculation (Fig. 75). GMCSF, C3 and SAA3 are all reported to play a role in the early phase of inflammation and aid in the recruitment of leukocytes. The complement system is involved in opsonization and lysis of microorganisms or recruitment of phagocytes to site of infection. The C3 is a key molecule in the complement cascade, its resulting cleavage products are involved in chemotaxis and opsonization. In this study, a sharp upregulation of C3 with a peak of 16.25 fold increase at 2 hours post innoculation was observed.

In *S. agalactiae* induced infection, the enhanced expression of GM-CSF and C3 could have lead to proliferation of granulocytes and monocytes, which is reflected by the upregulation of CD14, a cell surface marker protein expressed mainly by macrophages and neutrophils. There was also an enhanced expression of IL-12 which peaked at 24 h post infection. IL-12 has been demonstrated to upregulate other cytokines including TNF-α, IL-8 and IL-10 and also plays a critical role in altering the balance between Th1and Th2 responses. As the highest level of expression of IL-12 was found at 24 hrs post infection and as the experimental time period was not extended beyond 24hrs, the further course of other cytokines could not be detected. Interestingly, TLR4 and not TLR 2 was found to be upregulated, taking peak at fourth hour post infection.

On comparison of the extents and kinetics of the immune responses elicited by *S. aureus* (t6877) or *S. agalactiae* (VC394), it was observed that the differential responses were generated according to the strain of bacteria, implying alternative activation pathways

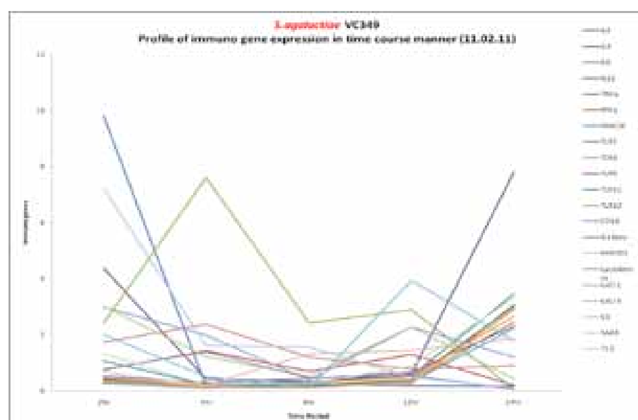


Fig. 75. Alteration in the mRNA concentrations of 21 immuno regulatory genes in mammary tissue after challenge with *Streptococcus agalactiae*. Shown are values for mean fold induction at times after challenge relative to the mRNA concentration measured for unstimulated cells.

or different levels of signal transduction. Differences were observed in the intensity of responses. Indeed, *S. aureus spa* type t6877 induced a greater expression of inflammatory mediators than *S. agalactiae* ST-483 initially but the expression declined very early. The histopathological studies of the tissues infected with these pathogens also revealed a higher degree of inflammatory and necrotic changes on IMI induced by *S. aureus* (t6877) than *S.agalactiae* (ST483). The profiling of cytokine expression can provide clue of disease outcomes and also the differential cytokine expression may have diagnostic application which may aid in the treatment of bovine mastitis. Further studies employing flow cytometry is underway to better understand the differential responses of the various immuno-modulatory cells in the mammary gland on intramammary infection by these pathogens.

Project : 19

External Funded Project under Public Private Partnership (PPP): Incidence of infection due to IBR and Leptospira in Indian Dairy Farms

S.S. Patil., K Prabhudas, M.R. Gajendragad, N.L. Gangadhar, V. Balamurugan and Divakar Hemadri

"Incidence of Infection due to Infectious Bovine Rhinotracheitis and Leptospira in Indian Dairy Farms" under PPP mode that was conceptualized, approved and launched during December, 2009. This is a unique and exhaustive study that included 11 organized dairy farms covering six states of India viz., Pondicherry, Tamil Nadu, Karnataka, Andhra Pradesh, Maharashtra and Chattisgarh. It is a comprehensive study inclusive of cattle, buffaloes maintained both by government and private sectors. It is evident from the study that the overall incidence of IBR and leptospirosis in Indian dairy farms was 42% and 20%, respectively. The outcome of the study has revealed that the incidence of IBR and leptospirosis in animals having reproductive issues was 54% and 22%, respectively. It is noteworthy to mention that the apparently healthy animals showed overall incidence of IBR and leptospirosis as 34% and 19%, respectively. The findings also revealed the overall incidence of IBR and leptospirosis in buffaloes as 31% and 20%, respectively and the single largest group of pathogenic leptospires isolated in this study was Leptospira Hardjo. The incidence data depicted here is more realistic as the study has covered 11 farms in six states. The economic impact and healthy carrier status of IBR/leptospirosis is well discussed in this study and is a matter of concern to animal and public health.

Analyses of the work done on Leptospira isolation and their identification by partial RNA polymerase beta subunit (*rpoB)* gene sequence analyses. It is evident that some of the Leptospira isolates were characterized to serovar level while some to serogroup or species level. It was found that *Leptospira borgpetersoni* Hardjo (12 isolates) was the single largest group of pathogenic Leptospira typed to serovar level (5%). It is quite interesting that there is an emergence of *L. inadai* as the major species among the isolates that accounted for 87 %. However, significance of *L .inadai* in bovine reproductive issues and public health is not clearly established anywhere in the world and the present finding requires further in-depth study. The significance of *L. inadai* in public health and animal reproduction is neither clearly understood nor documented much in literature. There is a suggestion that *L. inadai* are a group of bacteria that are believed to be in an intermediate species either pathogenic or non-pathogenic. The study has also revealed that only representative emergence of other pathogenic Leptospires in the interrogans species group (7 %). With *Leptospira borgpetersoni* Hardjo predominating as the most common incriminating Leptospira in reproductive problems and public health all over the world, as also in India as demonstrated in this study. At present an appropriate immunoprophylaxis incorporating this organism will be highly beneficial in offsetting losses to the farmers and dairy industry in the country.

Project Director's Report on AICRP

Centerwise summary

Ahmedabad (Gujarat)

- The state has unit has reported outbreaks of HS, FMD, PPR, CCPP, BQ and cowpox/buffalopox during 2010-11.
- Haemorraghic Septicaemia outbreaks were reported from Junagarh, Kutch-Bhuj, Rajkot, Mehsana, Banaskantha, Panchmahals, Ahmedabad in that order.
- Eight FMD outbreaks, two each in Mehasana, Panchmahals, Gandhinagar and one each in Banaskantha and Kheda were reported. Outbreaks in Mehasana and Banaskantha districts needs to be investigated thoroughly as these are covered under FMD-CP.
- Three outbreaks of PPR in Bhavangar, Junagarh and Valsad recorded.
- Of the five outbreaks of CCPP, four were reported from Bhavanagar and 1 from Porbander.
- Four outbreaks of BQ, in one each in Tapi, Valsad and two in Rajkot were recorded.
- One outbreak of cowpox/buffalo pox has also been reported.
- The coordinating unit also screened serum samples for FMD, PPR and breeding bulls and humans for Brucellosis using various ELISA formats. Two of the 240 breeding bulls and 23 of the 30 humans samples were positive for Brucellosis. About 18 and 23% serum samples were positive for FMD and PPR infection.
- Besides the above other lab investigations done at the unit are also reported.

Bengaluru (Karnataka)

- The unit had participated and carried out the sample based state level population survey of important diseases of livestock.
- The scientists from the Collaborating unit of ADMAS, Bengaluru along with the scientists working in the Eight Animal Disease Diagnostic Laboratory and Information centres investigated the reported disease outbreaks. A Total 130 outbreaks were investigated which are as follows: Haemorrhagic septicaemia: 20 outbreaks, Black quarter: 7 outbreaks, Enterotoxaemia: 7 outbreaks, Anthrax: 3 outbreaks, sheeppox & goatpox: 14 outbreaks, Pestes des petits ruminants: 13 outbreaks, and others including poultry 48 outbreaks.
- 215 outbreaks of Black Quarter were reported in the state and is an important bacterial disease among bovines. The disease was reported in 18 out of 30 districts, While Hassan district topped the list with highest number of 135 outbreaks during the year, Chikkaballapur, Dharwad, Gulbarga and Uttara Kannada districts were at the bottom, with one outbreak each district. The crude incidence rate of the disease during the period under report is 7.34 per 10^5 susceptible population, and crude mortality rate is 3.86 per 10^5 susceptible population. The case fatality rate observed during the year is 50.17%.
- One Hundred and Sixty outbreaks of Hemorrhagic Septicemia were reported in the state. The disease was reported in 15 out of 30 districts, While Chitradurga district topped the list with highest number of 56 outbreaks during the year, Bengaluru (U) district was at the bottom, with one outbreak. The crude incidence rate of the disease during the period under report is 9.89 per 10^5 susceptible population, and crude mortality rate is 3.78 per 10^5 susceptible population. The case fatality rate observed during the year is 36.77%.
- Sixty two outbreaks of Enterotoxaemia were reported in the state during the year. The disease is commonly reported from the sheep rearing districts in the state. Tumkur (27), Chitradurga (19), Gulbarga (5) have reported the disease outbreaks in the decreasing order. The crude incidence rate of the disease during the period under report is 9.32 per 10^5 susceptible population, and crude mortality rate is 5.13 per 10^5 susceptible population. The case fatality rate observed during the year is 48.96%.
- Fifteen outbreaks of Anthrax were reported in the state during the year. The number of outbreaks reported per month is within 5, the severity of the disease was greater during the month of of December 2010. The crude incidence rate of the disease during the period under report is 1.37 per 10^5 susceptible population, and crude mortality

rate is 1.24 per 10^5 susceptible population. The case fatality rate observed during the year is 90.90%. The disease is highly endemic in Bengaluru (R), Chamarajanagar, Chikkaballapur, Davanagere, Kolar and Tumkur districts.

- Seventy two outbreaks of Sheeppox and Goatpox were reported in the state during the year. The disease was reported from 13 out of 30 districts in the state. The most affected districts were Gulbarga (12), Bagalkot (11) Chitradurga (10) and Tumkur (8).The crude incidence rate of the disease during the period under report is 13.71 per 10^5 susceptible population, and crude mortality rate was 3.38 per 10^5 susceptible population. The case fatality rate observed during the year is 24.67%.
- PPR disease was reported from 9 out of 30 districts in the state. During the year under report 37 outbreaks were reported. The crude incidence rate of the disease was 8.54 per 10^5 susceptible population, and crude mortality rate was 1.38 per 10^5 susceptible population. The case fatality rate observed during the year was 11.45%.
- Economic analysis was done for diseases such as HS, BQ, Anthrax and ET.

Barapani (Meghalaya)

- Two major viral (FMD and CSF) and two major bacterial diseases (HS & BQ) were recorded in Meghalaya. Maximum number of outbreaks were reported during February (28), March (40). 2203 animals were affected in 194 outbreaks of FMD.
- Eighteen outbreaks of CSF affecting 223 pigs and killing 119 of them was reported. Maximum outbreaks of classical swine fever in swine of Meghalaya was reported during December (11), January (12), February (12) and March (18)
- Twenty four outbreaks each of HS and BQ affecting 240 animals and 351 animals were reported. Outbreak of BQ and HS were higher during the months of December, January and March.
- A total of 35 samples were collected from Yak from west Kameng district of Arunachal Pradesh for screening Brucellosis and IBR. None of the samples was found positive for brucellosis by RBPT while 18 samples were positive for IBR by indirect ELISA kit (IBR).
- Economic analysis was done for FMD, HS and BQ and swine diseases.

Bhopal (MP)

- The state has unit has reported outbreaks/ incidences of HS, FMD, PPR, BQ, rabies and classical swine fever during 2010-11.
- Thirty FMD outbreaks were reported from 10 districts of the State. Betul and Bhopal districts had maximum no. of outbreaks and seasonal trans-border migration of animals along the established cattle routes have been attributed to the outbreaks. Maximum no of outbreak were reported in month of March followed by winter months.
- Seventeen PPR outbreaks were reported from 9 districts of the State with maximum no. of outbreaks in Shivpuri district. Most of the outbreaks occurred in March and February months.
- Eight outbreaks CSF were reported from 06 districts in the State with most outbreaks reported from Chhindwara District. The outbreaks occurred in August, June and July.
- Incidences of Rabies were reported in Betul, Bhopal, Ashoknagar and Sagar districts. Betul district had a maximum no of incidences and the areas where the incidences were reported were located mostly in forest.
- A total 25 outbreaks of HS were reported from 13 districts of the State. Jabalpur district had maximum no. of outbreaks followed by Balaghat and Betul, Indore, Dhar, Katni districts. The maximum no. of outbreaks were reported in August month followed by April, May, June & July months.
- Five outbreaks of Black Quarter were reported from Chhindwara and Balaghat districts and one outbreak of Anthrax was reported from Sidhi district.
- Besides the disease reporting and investigation, the unit also carried out sero-surveillance for Brucellosis, double intra dermal test (DID) for TB in Govt. Breeding farms, private farms and villages and found most animals negative for tuberculosis. The unit also screened large number of samples for protozoan diseases and per cent positivity varied from 2.13 to 3.53.
- Economic analysis for FMD, PPR, Swinefever, HS, BQ and Anthrax and some parsistic diseases done.

Cuttack (Orissa)

- The important diseases of small ruminants in the state are PPR and CCPP with very high reported outbreaks and mortality every year. The other diseases of importance include goat pox, HS and enterotoxaemia. In bovines the outbreaks of B.Q. tops the list followed by H.S and Anthrax.
- 37 Black Quarter outbreaks have occurred in bovines in which 286 animals were affected and 206 have died. Highest number of outbreaks was reported in East and South Eastern coastal plain (23 OBS)
- Heamorrhagic septicaemia was recorded in Cattle, Buffalo, Sheep, Goat. A total of 14 outbreaks have occurred in bovines in which 136 animals were affected and 64 animals died. Maximum level of outbreak was observed in the month of July 2010 and January 2011. Two H.S. outbreaks occurred in Buffaloes in Deogarh and Gajapati districts in the month of October and February. Of the 27 affected animals 18 died. Two outbreaks of HS occurred in goats of Jajpur and Ganjam districts in the month of April, killing 21 of the 37 affected. H.S. outbreaks (2 OBS) was also observed in sheep in Jajpur and Khurda district in the month of April and August affecting 30 animals out of which 28 animals died. No outbreaks were recorded in the month of April, May, August, November, December and February which in directs less stress as well as due to regular vaccination. The outbreaks were recorded in the month of June, July, September, January and March.
- The prevalence of blood Protista and Rickettsia like Theilieriosis, Babesiosis, Trypanosomiosis and Anaplasma were studied and identified.
- The occurrence of different gastro-intestinal parasites like nematodes, trematodes and cestodes were identified.
- The prevalence of bovine, tuberculosis, john's diseases and brucellosis were studied confining to Government Livestock Breeding Farms as well as private farms.
- Sero-surveillance for Avian Influenza was carried out throughout the state.
- Extensively studied outbreaks with detailed economic analysis.

Guwahati (Assam)

- The unit collected sample based state level population survey of important diseases of livestock and poultry. Main livestock migratory Routes were identified.
- Meteorological data from 7 regional meteorological centres located in different districts of Assam were collected and the incidence of disease in the state was correlated.
- Out of five major bacteriological diseases of animals, outbreaks of haemorrhagic septicaemia occurred in highest numbers (166 outbreaks) and affected 22 districts. Maximum no of outbreaks were recorded from Barpeta, Dhemaji districts (12 each) followed by Goal Para and Golaghat district (11 each).
- Anthrax continues to be major disease of public health importance in four different districts of Assam.
- A total of 129 outbreaks of black quarter were recorded from 19 districts of Assam.
- Out of 743 serum samples tested for brucellosis, 74 were found to be positive. None was found positive for tuberculosis out of 231 animals tested by single intradermal tuberculin test.
- In case of viral diseases of animals, foot-and-mouth disease was found to be the major disease. Most of the out breaks of FMD were due to the serotype O.
- Among the viral diseases of pigs, 159 outbreaks of classical swine fever were recorded in 21 districts of Assam causing heavy morbidity and mortality.
- *Peste des petits ruminants* continues to be the emerging disease of goats in Assam and one out break was recorded in a government goat breeding farm in district of Assam after introduction of goats from other states of North India.
- Orf or contagious ecthyma is another emerging disease of goats in Assam and

  occurred in epidemic form in Golaghat and Kamrup districts. Out of 258 serum samples tested for orf, 208 samples were found to be positive.
- Besides the livestock diseases the state unit has also recorded many poultry diseases.
- Fascioliosis, amphistomiasis and babesiosis are other parasitic diseases recorded in the state.

Hyderabad (AP)

- FMD, PPR, Bluetongue, sheep and goat pox, rabies were the main viral diseases while HS, BQ, ET, Anthrax were the main bacterial diseases recorded in the state.
- Highest number of Hemorrhagic Septicemia outbreaks were recorded in Guntur district (28 outbreaks, 123 attacks, 40 deaths) followed by East Godavari district (20 outbreaks, 81 attacks, 19 deaths). Total Outbreaks were 78, with 369 attacks and 137 deaths. More number outbreaks (64 OBs) recorded in Coastal Andhra Region followed by Telangana (8) and Rayalaseema (6) regions. The disease is in decreasing trend when compared to previous years because of mass vaccinations conducted in all the endemic areas.
- Highest outbreak of BQ was reported in Visakhapatnam (8) followed by Adilabad (4). Region wise it is Coastal Andhra (9) with most outbreaks followed by Telangana (8) and Rayalaseem (1) regions._
- The incidence of Anthrax (Bovines) is in decreasing trend when compared to previous years due to wide spread vaccination program.
- Highest outbreaks of ET were reported from Anantapur, followed by Medak and Prakasam districts. The disease showed a reducing trend due to mass vaccinations in all endemic areas by campaign mode.
- PPR was reported in 11 districts of AP. Highest outbreaks were reported in Prakasam and Visakhapatnam districts.
- The incidence of Sheep pox disease was showing a decreasing trend and only 35 outbreaks were reported during the year, when compared to previous years. This may be due to protection of all susceptible sheep against Sheep pox in all the endemic areas of the districts every year by campaign mode.
- Blue Tongue was reported in three districts such as Medak, Krishna and Guntur and no incidence of the disease in the rest of the 20 districts. The disease incidence has decreased during the year 2010-11 when compared to previous years which may be due to herd immunity.
- A total of 4796 serum samples were examined for brucellosis out of which 536 samples are found positive (11.17%). During the year 2010-11, 1276 heads of cattle and 2628 heads of buffaloes have been screened against brucellosis. Out of which 74 cattle and 381 buffaloes have been found positive for brucellosis i.e. 4.88% in cattle and 9.75% in buffaloes. Brucellosis in human was also monitored by sero-prevalence studies. Around 180 human sera samples have been tested. Of which 30 samples were found to be positive for brucellosis i.e. 16.6%. This high percentage of incidence in humans might be due to retesting of positive samples repeatedly after each course of treatment.

Imphal (Manipur)

- Two FMD type O outbreaks; one at Imphal-West, affecting only 8 cattle of non descrpt local breed and one at Imphal-East affecting only three cattle of local breed without any mortality in both outbreaks.
- 14 outbreaks of Black Quarter outbreaks during the year; Imphal-East (7)and Imphal-West (4), Senapati (2) and Bishnupur (1) had outbreaks with a total of 207 attacks and 100 deaths. Maximum number of BQ outbreaks occurred during the month of September, 2010 and followed by February, 2011.
- There were 17 outbreaks of Classical Swine Fever with 245 attack and 120 deaths
- Two Heamorrhagic Septicaemia cases were reported at Imphal-West and Thoubal district of the state with 9 attack and 3 deaths.
- 980 cattle sera samples were screened by AB-ELISA for Brucella during the year under report of which 780 sera samples were tested at the ELISA Laboratory of the Unit and another lot of 200 sera samples were tested at the PD_ADMAS, Hebbal, Bengaluru.The results revealed the maximum prevalence of 98% in Imphal-East and minimum of 22% in Tamenglong district. All districts of the state had Brucellosis sero-positive cattle population.
- Of the 190 Goat and sheep sera samples screened for Brucella by PD_ADMAS, Hebbal, Bengaluru, only 3 goats in Senapati District and 1 in Thoubal District were seropositive.
- Similarly, the above sera samples were also tested for Infectious Bovine Rhynotrachietis(IBR) by

employing AB-ELISA Technique. Imphal-East had highest seropositive cases of 52% followed by 38% in Imphal-West and Thoubal district and a minimum of 8% seropositive in Ukhrul district.

- Surveillance of Bovine Tuberculin-PPD sensitive cattle of 6 districts (four valley and two hilly district) could not find out any sensitive reactors after 72 hours of observation.
- Likewise, intra-dermal sensitivity test of Jhonin-PPD in these animals could not find any positive reactor.
- Leptospires staining examination of 49 blood samples and 28 urine samples from febrile dogs, cats and goats by using PD_ADMAS Leptospira Staining kit did not show any positive reaction.
- Sero-surveillance of bluetongue in Goat, sheep and cattle was taken up in collaboration with All India Network Project on Blue Tongue, Kolkata Centre. Altogether, 176 goat and sheep sera samples were tested by iELISA and 37 sero-positive animals were detected. Similarly detection of the presence of Bovine bluetongue antibodies in cattle was also taken up by the Unit and 100 cattle sera samples-20 sera samples from five districts were screened and 21 positive animals were detected.
- Altogether, 1895 livestock faecal samples were examined as routine examination to detect parasitic load in these animals.
- Canine Rabies diagnosis was made for 8 positive cases in Imphal-East, Imphal-West and Ukhrul district of the state.
- Outbreaks of major poultry diseases in various parts of the state were also depicted in the separate table for poultry disease.
- 8 Training programmes for livestock farmers, members of NGOs, Dairy Federation/Societies and general public were also conducted by the Unit.

Jaipur (Rajasthan)

- In the year 2010-2011, Foot & Mouth Disease was the major disease that affected livestock in Rajasthan. The other disease outbreaks are as follows.
- In the total nine outbreaks of HS were reported affecting 114 and killing 68 animals. Jaipur district was most affected with 04 outbreaks (21 of 38 affected animals died). In Dholpur case fatality rate in the HS outbreaks was 100%. Other district affected were Alwar, Baran and Dausa.
- Three outbreaks of BQ was reported in Rajasthan. In the two consecutive previous years i.e. 2007-08 & 2008-09 2009-10 one outbreak was reported, However in the year 2006-07 there were two outbreaks of BQ one each at Ganganagar & Hanumangarh district.
- 223 samples (cow-178, buffalo-31, dog-1, camel-13) were screened for Brucellosis using Rose Bengal Plate Test (RBPT), of which 53 (cow-31, buffalo-22) were positive.
- Two outbreaks of PPR was reported from Kota & Baran districts in April 2010 & March 2011 respectively. In Kota district in one outbreak 60 goats were affected out of which 20 died.
- There were 3 outbreaks of classical swine fever affecting 202 animals of which 110 died due to disease showing about 55% mortality. Control of disease was achieved through effective immunization of animals.
- Out of 1672 samples screened, 25, 66,125 and 12 samples were found positive for theileriosis, babesiosis. trypanosomiosis and anaplasmosis respectively.

Kolkata (West Bengal)

- 23 anthrax outbreaks reported in this year are same as previous year. The morbidity rate (0.85%) and mortality rate (0.85%) has increased in comparison to previous year. Incidence reported was only from five districts.
- Eleven outbreaks of H.S have been reported in the West Bengal. The outbreaks show decreasing trend as compared to previous year. The case fatality rate (50%), Morbidity rate (1.18%) and Mortality rate (0.54%) were also decreased in comparision to previous years.
- The outbreaks of Black Quarter (100) increased remarkably in comparison to previous years. Morbidity (1.19%) and Case fatality rate (42.54%) increased but Mortality rate (0.50%) decreased.
- The number of PPR outbreaks (194) increased in comparison with last year. Case fatality rate (14.31%), Morbidity rate (11.63 %) and Mortality rate (1.66%) decreased. The highest number of

PPR outbreaks reported from Malda district (59), followed by Burdwan (18), Dakshin Dinajpur (12) and Birbhum (11) district

- Considerable increase in the number of sheep and goat pox outbreaks (127) with a about same case fatality rate (9.56%). In this year, incidence reported only from 9 (nine) districts. Highest incidence (35) reported from Hooghly, followed by fourteen (33) from Howrah, North 24 Paragana (23) and Burdwan (16) district.
- The outbreak of swine fever during last five year shows decreasing trend.

Ludhiana (Punjab)

- Two BQ outbreaks in a single farm in Patiala in October and December
- Two HS outbreaks in Ludhiana in May
- One outbreak of Sheep pox in migratory flock from Patiala
- Two outbreaks of Gastro enteritis in Pigs in Ropar and Ludhiana
- A total of 347 cattles were tested for Tuberculosis by SID (Single intradermal testing) and 30 animals (approx. 8.6%) were found positive. Out of total 72 goats tested for T.B (SID), 3 were found positive.
- A total of 347 animals were tested for Johne's disease and 4 animals were found to be positive.
- 2689 animals were screened for brucellosis (on the request of the farmers) and 775 animals were found positive on the basis of RBPT and STAT test. Large number of samples (244/367) Patiala were positive for Brucellosis. An overall increasing trend of Brucellosis over past five years was found.
- Ten tetanus incidences in Patiala
- Three cases of babesia in Kapurthala, Ludhiana and Moga, anaplasmosis in Kapurthala and Amritsar, theilariosis in Ludhiana were recorded.

Pune (Maharashtra)

- A total of 63 outbreaks of different diseases have occurred in large, small ruminants, pigs & poultry. In large ruminants, Black Quarter and Hemorrhagic Septicemia accounts for major losses whereas in small ruminants Hemorrhagic Septicemia and PPR predominates in disease outbreaks and causes major losses.
- The 13 outbreaks of Hemorrhagic Septicemia have occurred in bovines Killing59 of the 124 affected animals Incidence risk rate this year was higher compared to average of last five years (0.78%) and case fatality rate is same to that of average of last five years.
- There were 22 outbreaks of BQ in which 108 animals were affected and 81 have died. Incidence risk rate is higher as compared to average of last five year (0.27%) and case fatality is higher as compared to average of last five year (66.7 %).
- Incidence risk observed for anthrax during the year under report is 0.67% which is lower as compared to average of last five year (1.18 %). Case fatality rate is more as compared to average of last five year (23.87%).
- A total of nine PPR outbreaks have occurred in which 335 animals were affected and 100 animals were died. Incidence risk rate is lower as compared to average of last five year (3.98 %) and case fatality rate is higher (30%) as compared to average of last five year (23.47 %).
- Three outbreaks of sheep & goat pox have occurred in Kolhapur district in which 182 animals were affected and 93 animals were died. Incidence risk rate is overall same as compared to average of last five year (3.14 %) and case fatality rate is higher as compared to average of last five year (28 %).
- A total of 74 Pigs were affected and 65 have died in two CSF outbreaks. The Incidence risk rate is low as compared to average of last five year (21.1%) and case fatality rate is higher as compared to average of last five year (66.08%).

Srinagar (J & K)

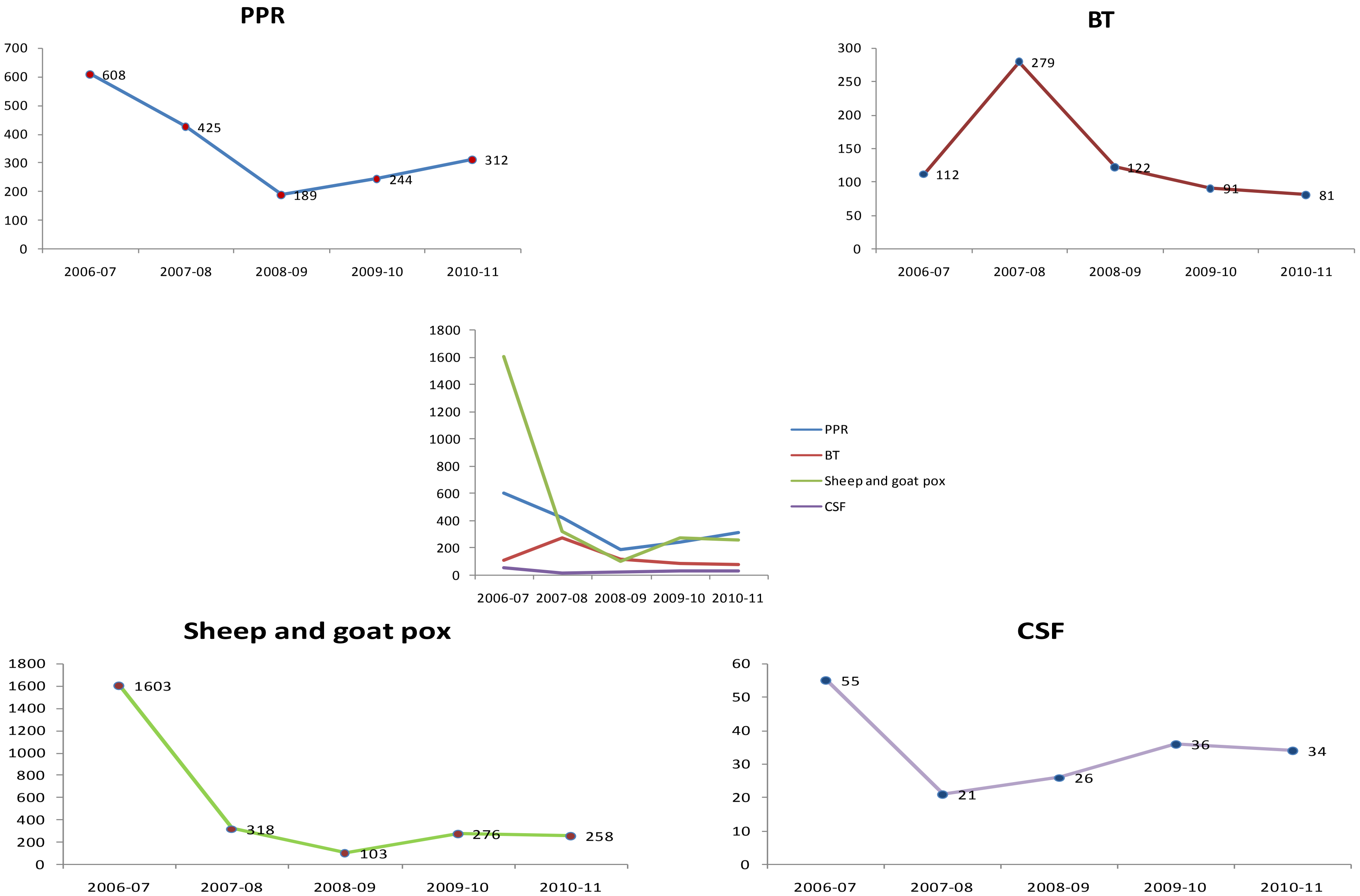
- Animal population survey in two villages each in Samba, Kathua, Reasi, Doda, Jammu and one village each in Pulwama, Udhampur, Kupwara completed.
- Ten animal diseases (Viral: FMD, Rabies, Sheep pox, PPR, Bluetongue, Equine Influenza; Bacterial: HS, BQ, Foot Rot and Anthrax) and four avian viral disease are regularly monitored by the Institute.
- FMD type O in Kulgam and Kargil districts of the state and 6 outbreaks in cattle, No sheep involved
- Four OB of HS Rajouri and Kathua affecting (31) cattle and causing death of (03) cattle September, November and December

- No anthrax, No TB by SIT
- 11 incidences of the Rabies disease were recorded in Kupwara and Kulgam districts of the state affecting (97) cattle with (05) deaths.
- 486 nos. of sera samples have been collected randomly from identified villages and were sent to PD-ADMAS Bengaluru for IBR and Brucellosis; the results are awaited
- 20 outbreaks of sheeppox, maximum outbreaks were mostly recorded in high snowfall areas, disease in all seasons.
- 10 outbreaks of footrot in early monsoon followed by late monsoon, mostly in hilly and high rainfall areas of Kashmir Division
- Economic analysis of two diseases (FMD and Sheep pox) provided.

Trivendrum (Kerala)

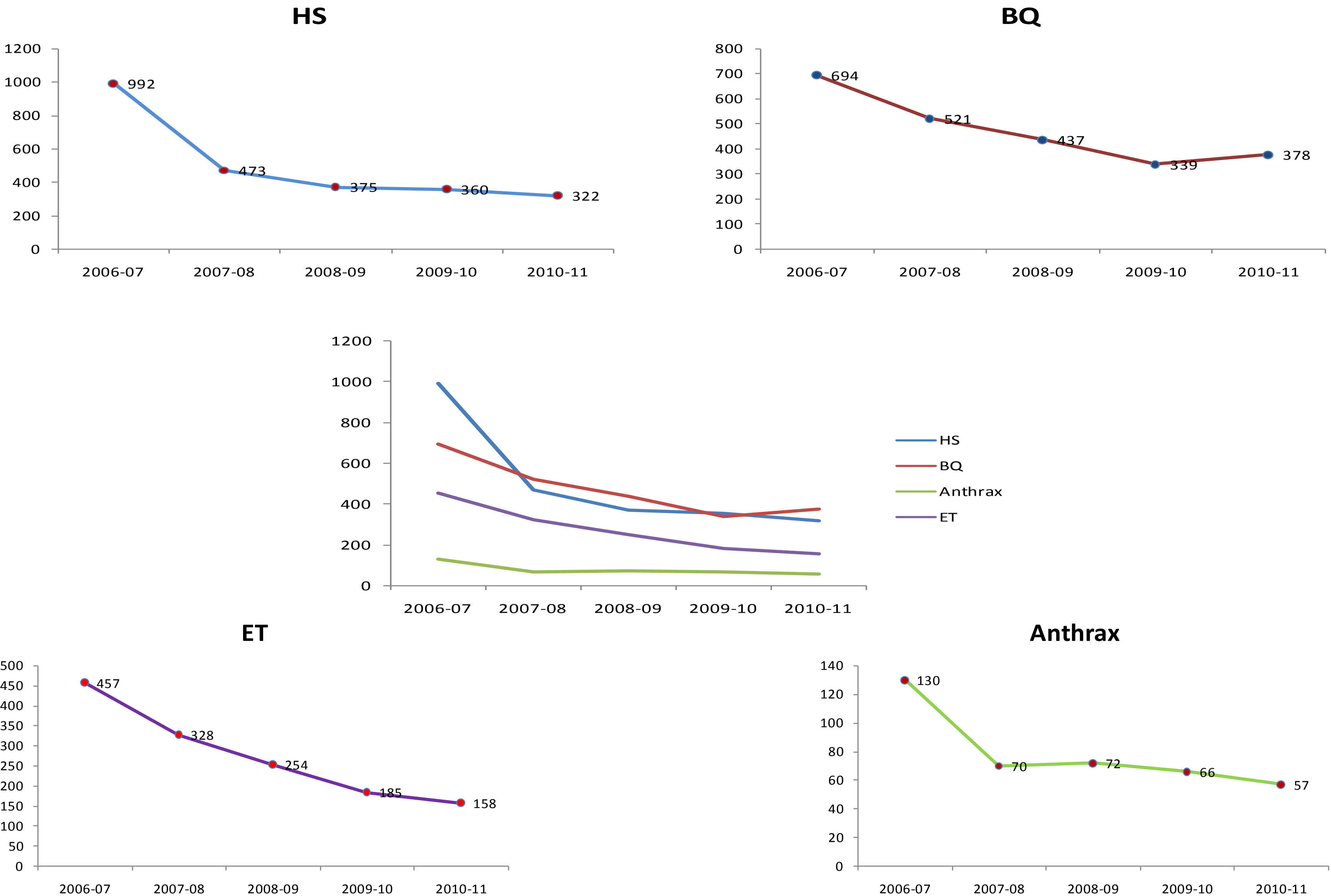
- Kerala has reported 42 outbreaks of FMD, five outbreaks of anthrax,three outbreaks of HS, one outbreak of PPR and 26 incidences of rabies, besides some parasitic diseases in the year 2010-11. No outbreak of classical swine fever was reported.
- Five outbreaks were reported in four districts, viz., Kollam, Pathanamthitta, Kannur and Thrissur during December 2010, February 2011 and March 2011. Six cattle were affected and the mortality was 100 %.
- Three outbreaks of HS with 6 attacks in cattle and 5 attacks in buffaloes were reported. Out of the 11 affected animals, 6 cattle died. Ernakulam and Kollam were the affected districts. A combined infection of Anthrax and HS was reported in two cattle and one goat at Edamullakkal Panchayath of Kollam District. Vaccination programme was undertaken and the disease could be contained without further incidence.
- A single out break/incidence of PPR was reported in Kerala in Akathethara panchayat of Palakkad district. A single goat was affected and the incidence was effectively controlled by timely vaccination. In the previous year also the outbreak of PPR in Kerala was from Palakkad district.
- Twenty six cattle and eleven goats were affected with rabies and all the animals succumbed to death. These are the laboratory confirmed cases only.
- Eight crossbred Holstein Friesian cows were diagnosed of having anaplasmosis during the month of December 2010 in Idukki district which was confirmed by blood smear examination and also by PCR, out of which six animals died.

Trends on Viral disease outbreaks (2006-2011)



*X axis - Year; Y axis - No. of outbreaks

Trends on Bacterial disease outbreaks (2006-2011)



*X axis - Year; Y axis - No. of outbreaks

Miscellaneous

Publications/Presentations/Documentation:

(i) Papers in peer reviewed Journals:

Krishnamoorthy P, Balachandran C, Gajendragad MR, Prabhudas K. (2010). A case report of cavernous haemangioma in the liver of Wistar rat. *Indian J. Vet. Pathol.* **34**: 76-77.

Patil SS, Hemadri D, Shankar BP, Raghavendra AG, Veeresh H, Sindhoora B, Chandan S, Sreekala K, Gajendragad MR, Prabhudas K. (2010). Genetic typing of recent classical swine fever isolates from India. *Vet Microbiol.* **141**: 367-73.

Patil SS, Hemadri D, Veeresh H, Sreekala K, Gajendragad MR, Prabhudas K. (2011). Phylogenetic analysis of NS5B gene of classical swine fever virus isolates indicates plausible Chinese origin of Indian subgroup 2.2 viruses. *Virus Genes.* PubMed PMID: 21246270.

Sengupta PP, Balumahendiran M, Suryanaryana VVS, Raghavendra AG, Shome BR, Gajendragad MR, Prabhudas K. (2010). PCR-based diagnosis of surra-targeting VSG gene: Experimental studies in small laboratory rodents and buffalo. *Vet. Parasitol.* **171**: 22–31.

Shome R, Gangadhar NL, K. Narayana Rao, B R Shome, K. Prabhudas (2011). Diagnosis of brucellosis in the equines by serological tests and PCR: A clinical report. *Indian J. Anim. Sci.* **81**: 137–139.

Shome BR, Shome R, Bujarbaruah KM, Das A, Rahman H, Sharma GD, Dutta BK. (2010). Investigation of haemorrhagic enteritis in pygmy hogs (*Sus salvanius*) from India. *Rev. sci. tech. Off. int. Epiz.*, **29**: 687-693.

Shome R, Bhure SK, Gangadhar NL, Shome BR, Deivanai M, Prabhudas K. (2011). Concurrent occurrence of *Leptonema* in *Brucella* infected veterinarian: A case report from India. *Indian Vet. J.* **88**: 16-17.

Shome R, Deivanai M, Shome BR, Rao NK, Prabhudas K. (2011). Isolation, serological and PCR confirmation of *Brucella suis* abortion in swine. *Indian Vet J.* (In Press).

Presentation in Conferences/ Symposia/ Seminars/ Other fora:

Krishnamoorthy P, Sengupta PP, Gajendragad MR, Prabhudas K (2010). Pathology of experimental *Trypanosoma evansi* (canine isolate) infection in Wistar albino rat. In: *National symposium on "Recent trend in diagnosis and pathology of emerging and re-emerging disease of livestock and poultry"*. 25-27 November, 2010, College of Veterinary Science, Guwahati, Assam, India. pp: 182.

Krishnamoorthy P, Rao SBN, Dinesh Kumar D, Prabhudas K (2010). Pathology of neem seed cake as protein supplement of feed in experimental Wistar albino rats. In: *National symposium on "Recent trend in diagnosis and pathology of emerging and re-emerging disease of livestock and poultry"*. 25-27 November, 2010, College of Veterinary Science, Guwahati, Assam, India. pp: 185.

Balamurugan V, Krishnamoorthy P, Veeregowda BM, Sen A, Rajak KK, Bhanuprakash V, Gajendragad MR and Prabhudas K (2010). Sero-epidemiological study of *peste des petits ruminants* in cattle and buffaloes in Southern India. Abstract presented in XVII annual convention of Indian Society for Veterinary Immunology and Biotechnology and international symposium on "Role of biotechnology in conserving biodiversity and livestock development for food security and poverty alleviation" held during 29-31st December, 2010 organized by Department of Veterinary Microbiology & Biotechnology, College of Veterinary and Animal Sciences, Rajasthan University of Veterinary and Animal Sciences, Bikaner – 334001, Rajasthan, India.

Balamurugan V, Gangadhar NL, Dutta M, Nagalingam M, Shome R, Krishnamoorthy P, Gajendragad MR and Prabhudas K (2010). Identification and characterization of the leptospira isolates from livestock and human. Abstract presented in XVI annual convention of Indian Society for Veterinary Immunology and Biotechnology and National symposium on Novel biotechnological and immunological interventions in mitigation of climate changes on production and protection of livestock and poultry held during 8-10th April, 2010 organized by Dept. of Veterinary Microbiology, Veterinary college and Research Institute, Namakkal, TANUVAS, TamilNadu,

Uma S, Narayanaswamy HD, Suryanarayana T, Gajendragad MR and Satyanarayana ML (2010) Molecular identification of *Mycoplasma agalactiae* isolated from sheep. Paper presented at the XXVII Annual Conference of Indian Association of Veterinary Pathologists held at College of Veterinary Science, Khananpara, Guwahati, November 25-27, 2010.

Gajendragad MR (2011) NADRES Now & *Next,* Presented at the brain storming session on animal disease monitoring and surveillance held at New Delhi on 25th January 2011.

Shome BR, Krishnamoorthy P, Shome R, Velu D, Bhuvana M, Krithiga N, Mitra S, Prabhudas K. (2010) *Staphylococcus aureus* (*spa* type t6877) induced experimental mastitis in mouse model. Presented in XXVII Annual conference of Indian Association of Veterinary Pathologists. Held in College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati-781022, and Assam during 25-27th November, 2010.

Shome R, Deivanai M, Narayana Rao NK, Nagalingam M, Shome BR, Prabhudas K. (2011) *Brucellosis: New demands for diagnostic innovations to aid public health in India* in "One Health 2011 Congress" Melbourne Convention Centre, Cnr Spencer & Flinders Streets Melbourne VIC 3000 Australia from 14 to 16 February 2011.

Shome R, Nagalingam M, Balamurugan V, Prashant G, Rao NK, Assabi SR, Shome BR, Prabhudas K. (2011) An investigation of zoonotic Brucella and Leptospira infections in a pig farm of Karnataka, India IX Annual Conference of Indian Association of Veterinary Public Health Specialists & *National Symposium on* "Veterinary Public Health: New Horizon for Integrating the Animal Production, Food Safety and Human Health" (28th & 29th January, 2011) Organized by Department of Veterinary Public Health Bombay Veterinary College, Parel, Mumbai- 400 012.

Shome BR (2011) Diversity of *Staphylococci* among the Bovine Mastitis isolates in India in Symposium on "Recent developments in *Staphylococcus aureus* biology and infections" held during 15th-16th January 2011 at Sir Dorabji Tata Centre for Research in Tropical Diseases, IISc Campus Bengaluru.

Training/Refresher Course/Summer/Winter School/Seminars/Conferences/ Symposia/Workshops/Programmes attended

Name of the Seminar/Workshop/ Training	Venue	Date	Scientist Attended
National Symposium on Photobiology of poultry and Laboratory Disease	Vet. College & RES Institute Namakal, T.N.	5- 6 March, 2010	Dr. M. R. Gajendragad Dr. P. Krishnamoorthy
XVI Annual Convention of Indian society for veterinary immunology and Biotechnology (ISVIB)	Veterinary collage and research Institute Namakal Tamil Nadu	8- 10 April, 2010	Dr. V. Balamurugan Dr. M. Nagalingam
17th Annual Meeting of AICRP units of PD_ADMAS	College of Veterinary Sciences, Assam Agricultural University, Guwahati, Assam	23-24 April, 2010	Dr. K. Prabhudas Dr. M. R. Gajendragad Dr. B. R. Shome Dr. R. Shome Dr. P. P. Sengupta Dr. P. Krishnamoorthy Dr. Mohd. Mudassar Chanda Dr. J. Hiremath Dr. M. Nagalingam
Preparation of SOP/Action plan for responding to terrorist attack using biological agents.	DG/IG Police, Govt of Karnataka	27 April, 2010	Dr. D. Hemadri
Annual Review Meeting of the All India Network Project on Bluetongue	College of Veterinary Sciences, TANVASU	4-5 May, 2010	Dr. D. Hemadri
Brucella Network Project Meeting conducted by DBT	DBT, New Delhi	29 May, 2010	Dr. Rajeswari Shome
Half yearly review meeting of Outreach programme on zoonotic diseases	Veterinary College Mumbai	11 June, 2010	Dr. M. Nagalingam
"Installation and orientation training programme for SAS software"	University of Agricultural Sciences, GKVK, Bengaluru	21-22 June, 2010	Dr. P. Krishnamoorthy
14th Management development programme in Agricultural Research	National Academy of Agricultural Research management, Hyderabad	22- 27 July, 2010	Dr. M. R. Gajendragad
"SAS : an overview"	Indian Agricultural Statistics Research Institute, New Delhi	17-22 August, 2010	Dr. P. Krishnamoorthy
Introduction to GIS and its Applications	National Remote Sensing Center, Hyderabad	30 August - 24 September, 2010	Dr. D. Hemadri
Hands on Workshop on Microarray Technologies	Manipal life science center, Manipal	18-23 September , 2010	Dr. Mohd. Mudassar Chanda
18th Annual Meeting of AICRP units of PD_ADMAS	PD_ADMAS, Bengaluru	12-13 November, 2010	Dr. K. Prabhudas Dr. M. R. Gajendragad Dr. B. R. Shome Dr. D. Hemadri Dr. R. Shome Dr. P. P. Sengupta Dr. V. Balamurugan Dr. S. S. Patil Dr. P. Krishnamoorthy Dr. Mohd. Mudassar Chanda Dr. J. Hiremath Dr. M. Nagalingam

Name of the Seminar/Workshop/ Training	Venue	Date	Scientist Attended
Midterm review meeting of All India Network Project on Bluetongue	NASC, NewDelhi	19 November, 2010	Dr. D. Hemadri
XXVII Annual Conference of Indian Association of Veterinary Pathologists	Department of Pathology, College of Veterinary Science, Guwahati	25-27 November, 2010	Dr. M R Gajendragad Dr. B R Shome
ICMR Centenary International Seminar on Atypical Clinical Presentations of Leptospirosis, Pathophysiology and Serovars and Genotypes involved	Regional medical Research centre (ICMR) Port Blair	1 December, 2010	Dr. V. Balamurugan Dr. M. Nagalingam
ICMR Centenary International Work shop on "Laboratory Diagnosis of leptospirosis and serological and molecular characterization of leptospirosis"	Regional medical Research centre (ICMR) Port Blair	2-8 December, 2010	Dr. V. Balamurugan Dr. M. Nagalingam
3rd Bengaluru Nano Conference on "Frontiers of Nanotechnology: Impact on India"	The Lalit Ashok, Bengaluru	8-9 December, 2010	Dr. B. R. Shome
National Conference of Biotechnology, Bioinformatics and Bioengineering	Dhramapuri	17-18 December, 2010	Dr. S. S. Patil
Data analysis using SAS Strengthening Statistical computing for NARS	UAS, GKVK, Bengaluru	17-22 January , 2011	Dr. Mohd. Mudassar Chanda Dr. Jagadish Hiremath
Midterm review meeting of Outreach programme on Zoonotic Diseases	NASC, NewDelhi	18 January, 2011	Dr. Rajeswari Shome
National Consultation on Animal Disease Monitoring and Surveillance in India	NASC, New Delhi	25 January, 2011	Dr. K. Prabhudas Dr. M. R. Gajendragad Dr. B. R. Shome Dr. D. Hemadri Dr. P. P. Sengupta
NAIP Component-4: Thematic Annual Workshop 2011 on "Basic and Strategic Research in Frontier Areas of Animal Science"	NDRI, Karnal	7-8 February, 2011	Dr. B. R. Shome
NAIP Component-4: Review meeting on performance of consortia	GB Pant University of Agril and Technology, Pantnagar	18-19 February, 2011	Dr. B. R. Shome
"Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA) 2011"	CPCSEA, Chennai	1-2 March, 2011	Dr. P. Krishnamoorthy
National Symposium on "Microbial Diversity and is applications in health, Agriculture and Industry"	ICAR Research Complex for Goa, Old Goa	4-5 March, 2011	Dr. B. R. Shome

Awards/Fellowships/ Recognition

Dr. P. Krishnamoorthy as scientist member of team got **Indian Council of Agricultural Research (ICAR) award for Outstanding Interdisciplinary Team Research in Agriculture and Allied Sciences** at ICAR awards ceremony held on 16th July 2010 at National Academy of Science Complex, New Delhi.

Dr. Jagadish Hiremath has been awarded ICAR international fellowship programme for pursuing PhD on **"Molecular Diagnosis of Genetically modified Influenza viruses and Mucosal Vaccine development against Influenza"** at Ohio State University, USA.

DR. Mohd.Mudassar Chanda awarded the studentship to pursue PhD on "**Understanding the epidemiology and impacts of bluetongue virus in South India with statistical and biological models"** at Oxford University and Centre of Ecology and Hydrology, Edinburgh. The project is part of joint **BBSRC/DFID/Scottish Initiative "Combating Infectious Diseases of Livestock for International Development" and All India network project on Bluetongue AINP-BT, ICAR (Government of India).**

Dr. B. R. Shome has been awarded **Fellow of Indian Association of Veterinary Public Health Specialists (IAVPHS) f**or the Advancement of Veterinary Public Health at National Symposium on "Veterinary Public Helath: New Horizon for Integrating the Animal production, Food Safety and Human Health" held on 28th & 29th January 2011 at Veterinary College, Mumbai.

Dr. S. S. Patil has been awarded **Fellow of Society for Applied Biotechnology** during National Conference of Biotechnology held in Dharmapuri (TN), 17-18 December 2010.

Dr. Divakar Hemadri has been nominated as Sectional Editor of Indian Journal of Virology published by Springer.

Dr. P. Krishnamoorthy has been nominated as Reviewer for the Indian Journal of Animal Sciences published by Indian Council of Agricultural Research (ICAR), New Delhi.

Dr. Rajeswari Shome, awarded DST-travel grant to attend "One Health Conference" held during 11-14th Febuary 2011 at Melborne, Australia

Distinguished Visitors

Dr. S. Ayyappan, Secretary DARE and DG, ICAR, NewDelhi
Dr. K. M. L. Pathak, Deputy Director General (AS), ICAR, NewDelhi
Dr. Gaya Prasad, Asst Director General (AH), ICAR, NewDelhi
Dr. C. S. Prasad, Asst Director General (ANP), ICAR, NewDelhi
Dr. Lalkrishna, Former Asst Director General (AH), ICAR, NewDelhi
Dr. R.N. Sreenivasa Gowda, Former VC, KVAFSU, Bengaluru
Dr. M. Rajasekhar, Former Project Director, PD_ADMAS
Dr. Leo Loth, FAO, Rome
Dr. David, Castellan FAO, Thailand
Dr. M. Moni, Deputy Director General, NIC, New Delhi
Dr. S. K. Bandyopadhyay, Sr. Tech. Co-ord. ECTAD
Dr. M. V. Subbarao, FAO Expert

Important Committees

Institute Management Committee

Name	Designation
Dr. K. Prabhudas	Chairman
Dr. S. Gopalkrishna	Member
Dr. Prakash Khandekar	Member
Dr. M. Rajasekhar	Member
Dr. S. N. Singh	Member
Dr. Gajendragad M. R.	Member Secretary

Recearch Advisory Committee

Name	Designation
Dr. R. N. Srinivasa Gowda	Chairman
Dr. Gaya Prasad	Member
Prof. K. Kumanan	Member
Dr. M. L. Mehrotra	Member
Dr. B. Pattanaik	Member
Dr. M. Rajasekhar	Member
Dr. S. N. Singh	Member
Dr. K. Prabhudas	Project Director
Dr. M. R. Gajendragad	Member Secretary

Quinquennial Review Team

Name	Designation
Dr. A.T. Sherikar	Chairman
Dr. A.K. Gehlot	Member
Dr. G.K. Singh	Member
Dr. R. Raghavan	Member
Dr. G. Butchaiah	Member
Dr. P.D. Juyal	Member
Dr. V.D. Sharma	Member
Dr. M. R. Gajendragad	Member Secretary

Staff position during 2010-11

S. No.	Name	Designation	Period from
	Dr. K. Prabhudas	Project Director	Oct, 2002
Scientific Staff			
1.	Dr. M. R. Gajendragad	Principal Scientist	Feb, 2006
2.	Dr. B. R. Shome	Principal Scientist	May, 2006
3.	Dr. D. Hemadri	Principal Scientist	Dec, 2008
4.	Dr (Mrs) Rajeswari Shome	Senior Scientist	Aug, 2005
5.	Dr. P. P. Sengupta	Senior Scientist	May, 2002
6.	Dr. V. Balamurugan	Senior Scientist	Jun, 2009
7.	Dr. S. S. Patil	Scientist (Sr. Scale)	Feb, 2006
8.	Dr. P. Krishnamoorthy	Scientist	Jan, 2008
9.	Dr. Mohd. Mudassar Chanda	Scientist	Jun 2009
10.	Dr. M.Nagalingam	Scientist	Aug, 2009
11.	Dr. Jagadish Hiremath	Scientist	Aug, 2009
Administrative Staff			
1.	Mr. S. R. Nataraj	Asst Adm Officer	Feb, 2007
2.	Mr. A. Srinivasamurthy	A.F. & A.O.	Aug, 2009
2.	Mr Rajeeva Lochana	Assistant	Apr, 2007
3.	Mr. N. Narayana Swamy	UDC	May, 2002
4.	Mrs. N. Padmini	LDC	Mar, 2007
Supporting Staff			
1.	Mr. M. K. Ramu	SSGr - I	Aug, 2007
2.	Mr. H. Shivaramaiah	SSGr - I	Sep, 2007
3.	Mr. B. Hanumantharaju	SSGr - I	Sep, 2007

Resource Generation

S. No.	Type of Activity	Units sold	Amount (in ₹)
1	Contract Research Project	One	5,40,000.00
2	Sale of IBR ELISAkits	Seven	2,36,700.00
3.	Sale of *Brucella* AB-ELISA kits	Eleven	
4	Sale of *Leptospira* staining kits	Thirteen	
5	Bench fee received for PG Research / Training		40,000.00
		Total	816700.00

Budget

Head of Account		Non-Plan ₹ (in Lakh)		Plan ₹ (in Lakh)	
		Allocation	Expenditure	Allocation	Expenditure
Recurring expenditure	Establishment charges	193.80	166.35	--	-
	Travelling allowance	0.50	0.495	7.00	6.53
	HRD	--	--	--	--
	Contigengies	38.50	35.89	--	--
	Sub Total (A)	**232.80**	**202.74**	**7.00**	**6.53**
Non-recurring	Equipments			50.00	50.00
	Works	2.50	2.50	260.00	260.00
	Vehicles	--	--	--	--
	Others/Books	--	--	33.00	30.66
	Sub Total (B)	--	--	--	--
	Grand Total (A+B)	**235.30**	**205.24**	**343.00**	**340.66**
Revenue receipts including TDR interest		₹ 6.40			
Interest earned on loans / advances		₹ 0.74			
Miscellaneous		₹ 3.98			

ADMAS News

Secretary DARE and DG, ICAR lays Foundation Stone for the Project Directorate on Animal Disease Monitoring and Surveillance (PD_ADMAS) at Bengaluru

Dr. S. Ayyappan, Secretary, Department of Agriculture Research and Education (DARE), Ministry of Agriculture, GOI and Director General, ICAR laid the foundation stone of the new campus of the Project Directorate on Animal Disease Monitoring and Surveillance (PD_ADMAS) at Yelahanka (Bengaluru) on 11th of November 2010. Dr. K M L Pathak, DDG (AS), Dr. Gaya Prasad, ADG (AH), Dr. C. S. Prasad ADG (APN), Dr. K. Prabhudas, Project Director, PD_ADMAS, Dr. Venkatarmanan, JD, IVRI- Bengaluru and Dr. R. N. Sreenivasa Gowda former Vice Chancellor, KVAFSU, Dr. Rajasekhar former Director, PD_ADMAS and other dignitaries and scientists of PD_ADMAS were also present on the occasion. To mark the occasion, saplings were planted in the campus by honourable DG, DDG (AS), ADG (AH) and ADG (ANP).

While speaking on the occasion Dr. S. Ayyappan congratulated the Project Director, scientists and staff of PD_ADMAS. In his speech, Hon'ble DG pointed out the relevance of the Institute especially in today's globalized world and role of PD_ADMAS in disease epidemiology and development of disease forecasting models. He was also glad to say that PD_ADMAS is a unique governmental mechanism, seen only in few countries. The successful functioning of this system can play a major role in the control and preventions of animal diseases in India.

The ceremony was concluded with vote of thanks by Dr. K Prabhudas.

Dr. H. Rahman takes over as Project Director

Dr. Habibur Rahman has taken over as the Project Director of PD_ADMAS on 30th April 2011. Though a trained veterinary microbiologist, Dr. Rahman has been known also for his research work beyound is field. He held many important research management positions such as Joint Director, ICAR Res. Complex, Gangtok, Head, Division of Veterinary Public Health, IVRI, Izatnagar, Head, Div. of Animal Health, ICAR Res Complex, Barapani etc before joining this institute. He has published more than 150 research articles in national and international scientific journals and has advised 21 Masters' and 12 PhD students in the area of Microbiology and Biotechnology. Dr. Rahman is the recipient of many prestigious awards including DBT Crest Award and Visiting Fellow Germany. The Editorial Committee of annual report 2010-11 and staff of PD_ADMAS wish Dr. Rahman all the best in his new responsibility.

PD_ADMAS bids farewell to Dr. K. Prabhudas

Dr. K.Prabhudas was born in Guntur district of AP. on 27.4.1949. He completed his BVSc. & AH and MVSc from Tirupati Vety, College , AP and Ph.D from IVRI. He joined ARS service in 17.9.1976. He served Mukteshwar and Bengaluru station of IVRI. He is one of the key person in control programme of FMD in the country. He underwent several training programmes in Switzerland under Indo-Swiss collaborative research project. He guided several Master's and Doctoral degree students. In 2002 he was given the charge of Project Director of PD_ADMAS, Bengaluru. In 2007 he was selected by ASRB as Project Director, PD_ADMAS. Till his superannuation he remained in this post. He remained functional for the implementation of different projects and planning of the proposed new building at Yelahanka, Bengaluru. Due to his helping attitude and polite behavior, he was very popular among scientists, researchers and other staff members. All the staff members of PD_ADMAS bid him farewell on 30.4.11. and they wish him a very happy and peaceful retired life.

PD_ADMAS bids farewell to Mrs. N. Padmini

Mrs. N. Padmini born on 2.4.1951. She joined ICAR service in 6.12.1980 as a Literate Grade I and promoted to the post of LDC on 30.8.1996. She served NDRI, Bengaluru till 28.2.2007 and joined PD_ADMAS on 1.3.2007. She was promoted to the post of UDC on 1.11.2010. She was very co-operative to all the staff of PD_ADMAS. She retired from her service on 30.4.11. All the staff members wish her also a very happy and peaceful retired life.

Institute's Field Activities