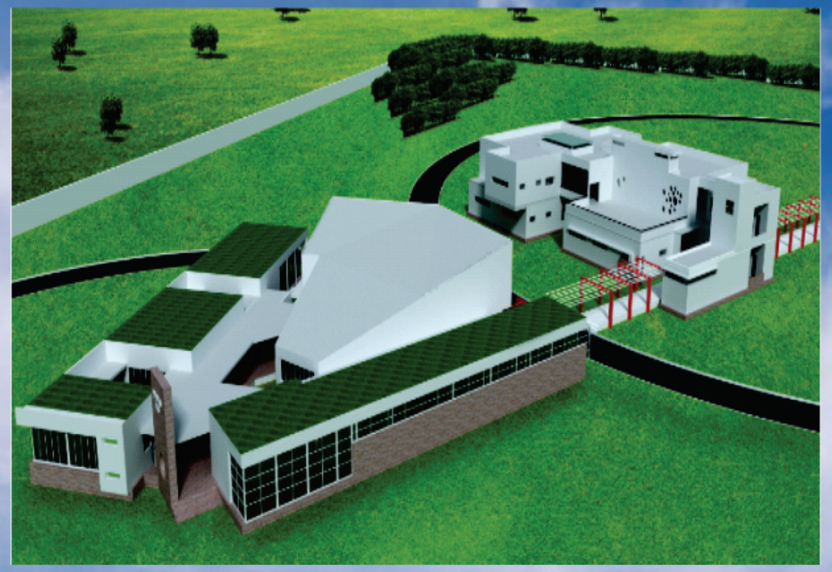

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Project Directorate on Animal Disease Monitoring and Surveillance

ANNUAL REPORT 2011-12

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Decadal trend of Anthrax occurrence in India (1991-2010)

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Proposed new building of PD_ADMAS at Yelahanka, Bengaluru

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I acknowledge all the help extended by the staff member of the Project Directorate.

Jai Hind

(H. Rahman)
Project Director

Executive Summary

Quantitative methodologies are being used to make predictions and forecasting about the occurrence of disease outbreaks, transmission of the disease and its control. Disease risks, demography and climate change are the ideal drivers to develop a holistic approach for quantitative modeling of epidemiology and control of livestock diseases. Different kinds of predictions are being used for modeling. The first kind deals with the risk that an exotic infection will appear in a livestock population. Second kind is that, in case of endemic diseases, how fast the disease transmits, how many number of animals will be affected and how long will it persists in the population? Last kind of prediction is the intervention models in relation to containment of the epidemic disease, what will happen if animals are vaccinated in an outbreak scenario and how quicky the disease can be controlled? Such questions are attempted to answer using the prediction models. A new dimension in the field of disease modeling is the need of hour. Quality database is the cynosure of all the prediction and forecasting frameworks. Strengthening of database is an ongoing activity and quality data is added to the database of the directorate each year and active surveillance of livestock diseases will surely enhance the quality of the database.

The livestock disease database has 90,104 records. These records have link to zone, state and district parameters of their respective states. Further, the data has been arranged species-wise, month-wise, district-wise. The data has been analyzed for disease ranking at national level and state level. They have also been ranked on etiological basis. Although the national cumulative disease ranking shows that HS, FMD and Babesiosis are the top reported diseases, during the year 2010 – 11, HS, PPR and ET are the top diseases reported in terms of incidence. There is a clear cut decline in FMD cases, however the other diseases viz., anthrax, Sheep and goat pox, BQ are being reported regularly. Amongst the parasitic diseases theileriosis and trypanosomiasis are recorded where as babesiosis was not recorded at all. Month-wise analysis of the data shows that diseases such as Anthrax, BQ, ET, FMD, HS, PPR and Sheep & Goat pox were reported throughout the year. This result shows that occurrence of many diseases do not adhere to any particular season.

During the year under report two major diseases, Anthrax and Haemorrhagic septicaemia were selected for detailed analysis.The epidemiology of anthrax (from 1991 to 2010) has been constructed based on the disease records available in National Animal Diseases Referral Expert System (NADRES). As per NADRES data, anthrax features as one of the top ten diseases reported in India and also as one of the major causes of death in livestock. Anthrax has been reported in eighteen states *viz.*, Andhra Pradesh, Assam, Bihar, Chhattisgarh, Gujarat, Himachal Pradesh, Jammu and Kashmir, Jharkhand, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Odisha, Rajasthan, Tamilnadu, and West Bengal during the last two decades (1991-2010).

There has been a constant variation in the percentage of states reporting anthrax (calculated as percentage of total states reporting disease data), but an overall increase was seen since 1999 onwards which might be due to the gradual spread of the disease. The occurrence of anthrax showed a progressive trend with a gradual increase in the number of outbreaks since 1992 with a peak seen between 2000 and 2002. From 2002 onwards, there is a gradual decline in the occurrence of anthrax. This could be attributed to awareness amongst the farming community and the control measures taken up by the authorities. In India, the occurrence of Anthrax varies greatly in different zones of the country as the climate and ecological factors varies greatly between the zones. Anthrax has been reported widely in the south zone of the country followed by east, west and north-east zones.

The epidemiology of HS (from 1991 to2010) has been constructed based on the disease records available in NADRES. HS features as the second most reported disease in India during the last two decades and is the cause of maximum number of deaths reported in livestock in the

country. HS has been reported in twenty five states *viz.,* Andhra Pradesh, Arunachal Pradesh, Assam, Bihar, Chandigarh, Chhattisgarh, Goa, Gujarat, Haryana, Himachal Pradesh, Jammu and Kashmir, Jharkhand, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Mizoram, Odisha, Punjab, Rajasthan, Tamil Nadu, Uttaranchal and West Bengal during the last two decades (1991-2010). A progressive trend was seen in occurrence of HS since 1992. Maximum outbreaks were reported in 1998. There was a gradual decline in the number of HS outbreaks reported since 1998. This could be attributed to the vaccination strategies followed by the different state governments.

The occurrence of HS has been reported in all the six zones of the country. High prevalence of HS has been reported in the south zone of the country followed by west, central, east, north and north-east zones. Though there was a lot of variation in the number of outbreaks reported across the years in the different zones, a peak was seen every three years and the trend of HS occurrence seemed to follow a similar pattern in south, west and central zone. The pattern of disease occurrence over the two decades is similar in east and north-east zones. These similarities could be due to the geography and meteorological conditions in these zones. India has different agro-climatic zones and the occurrence of the disease across the country varies greatly. Based on the 20 years' reports of HS occurrence, the very high, high, medium and low risk areas have been mapped. Most of the districts of Andhra Pradesh, Karnataka, Maharashtra, and Gujarat have been identified as very high HS pathozone belt. Among the states of south zone, Tamilnadu and Kerala have effectively contained the disease with proper vaccination and most of their districts fall under medium or low HS pathozone. There are pockets of very high HS pathozones throughout the country. In the East zone, many districts bordering the junction between the states of Bihar, Odisha and West Bengal belong to high HS pathozone. The decadal and quinquennial trend of HS occurrence are studied. Considerable variation in year to year occurrence of the disease is seen in different states. There is an overall decrease in the HS outbreak reports in the different states of the country during 2001-2010 compared to 1991-2000, except in Bihar, Odisha and Assam. On analysis of data at five year intervals between 1991 and 2010, highest occurrence was observed in the 1996- 2000 period in most states.

There has been a gradual decline in HS outbreaks since 1996-2000 and many states have reported fewer outbreaks during 2006-2010. The decline in the occurrence of HS in India can be attributed to the availability of cost-effective programmes for prevention and control. Many districts of Madhya Pradesh, Odisha and Rajasthan have reported the disease for the first time during the last 5 year study period. Newer districts reporting the disease can be viewed as a threat as it indicates spread of the disease.

The latest data collected as indicated earlier has been incorporated in the model and integrated to each district for each calendar month for a specific disease. A logistic regression analysis for 15 economically important livestock diseases is carried out. The model 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 using *MapInfo* software for all the 15 diseases. The latest forecasting maps are uploaded into the NADRES software accessible at www.nadres.res.in

Seroprevalence of *Peste des petits ruminants* (PPR) in cattle and buffaloes was carried out during the period using the randomly collected serum samples from different parts of Southern peninsular India. Analysis of 2,159 serum samples indicates an overall 4.58% prevalence of PPRV antibody in cattle and buffaloes. The true prevalence of PPRV antibodies in cattle and buffaloes were 5.21 and 4.82, respectively. The percentage prevalence of PPRV antibody in Karnataka, Tamilnadu, Puducherry and Andhra Pradesh were 4.23, 9.65, 4.32 and 1.3, respectively in bovines.

Classical swine fever (CSF) is one of the economically important diseases pigs in India.

Unfortunately, this disease has never got the attention it deserved due to the fact that major chunk of pig husbandry is mainly restricted to socially backward classes of the Indian society and also to the fact that major portion of the pig population is present in North Eastern states. A total of 154 CSF outbreaks were reported during the year 2011-12. North Eastern states accounted for most of the outbreaks with the state of Meghalaya being worst hit followed by Assam. Large number of outbreaks were reported from East Khasi Hills (70 nos) and West Garo Hills (35) was probably due to non vaccination of the pigs for the past two years and recent introduction of some pigs from unknown sources. A total of 426 pig serum samples from Andhra Pradesh (9), Karnataka (21), Odisha (5), Manipur (64), Maharashtra (22), Madhya Pradesh (254) and Rajasthan (51) were screened for the presence of antibodies against CSFV infection using competitive ELISA. All the pigs under this study were unvaccinated. A total of 191 sera samples were positive for CSF antibodies (percent positive was 44.83%). Movement route of pigs between Karnataka, Kerala and Tamilnadu were also studied.

A total of 2275 sera samples of bovine origin from 11 states were screened for IBR antibodies and 507 sera samples were found positive (percent positive was 22.7%). The highest prevalence was 48.7% (75/154) in Manipur and lowest was 2.58% in Karnataka (4/141). The variation in positive prevalence was due to sample size and not following the randomization techniques in collection of samples. The overall seroprevalence of IBR during 1995-2012 was found to be 35.85 %. In cumulative study 59284 serum samples from different parts of the country were tested by AB-ELISA during these years and 21256 samples were found positive. The variation in the overall prevalence of IBR may be attributed to the sample size.

The newly updated GIS maps of Karnataka and Tamilnadu are given here which shows updated districts within the circle. The bluetongue disease database for Karnataka presently contains information about 2500 outbreaks spanning the past 15 years.Bluetongue disease mapping for the state of Karnataka for the past 15 years was done. The map shows higher incidence of the disease in the southern region of the state, although the northern region has higher sheep population. The reasons could be vicinity of veterinary institution, progressive nature of the farmers in southern region, nomadic nature of the sheep farmers in the northern region. The map shows occurrence of the disease in 9 districts of the state, with highest number of outbreaks being reported from Tumkur district, followed by Chitradurga and Bagalkot districts.

During the year under report, some of the clinical samples collected from Chitradurga and Chikballapur districts between 2010 and 2011 were subjected to PCR. All the eight samples analysed were tentatively typed as serotype 2. Then the PCR products were subjected to restriction endonuclease analysis, which further confirmed that all the products indeed belonged to serotype 2. To support the above findings, the PCR products were nucleotide sequenced. So obtained nucleotide sequences were aligned with that of 23 other serotypes and used for construction of phylogenetic tree using neighbour joining method. From the genetic analysis, it was clear that the viruses from Chitradurga and Chikballapur are about 96-97% genetically similar to Mehboob nagar and Tirupati isolates and are different from South African reference and European isolates. Study also showed that source of infection for both the above outbreaks were different, although, both the isolates belonged to eastern topotype.

Phylogenetic analysis of 52 isolates based on *rpoB* gene sequences revealed that 31 isolates belong to either *L. borgpetersenii* or *L. interrogans* species, 13 isolates belong to *L. inadai* subgroup or subspecies and 8 isolates belong to *L. kirschneri* species. The overall observed prevalence of *Leptospira* species viz. *L. borgpetersenii/L. interrogans, L. inadai and L. krischneri* in animals and human are 59.62%, 25% and 15.38%, respectively. Based on the present study, the major circulating pathogenic species of *Leptospira* in animals and human were *L. borgpetersenii*, *L. interrogans, L. kirschneri*, *and L. inadai* subgroup.

Brucellosis is a bacterial disease caused by various Brucella species, which mainly infect cattle, swine, goats, sheep and dogs. Humans generally acquire the disease through direct contact with infected animals. A total of 2912 random sera samples from cattle (1056), buffaloes (332), sheep (180), goats (828) and swine (307) were received from 10 AICRP centers for brucellosis screening during the period. 363 out of 2915 (12.46%) samples were found positive ELISA. In the OPZD project, sera from risk group and livestock species from various farms were collected and tested for brucellosis. A total of 219 sera samples were collected from farm workers and veterinary doctors and screened for anti-*Brucella* antibodies. Nine out of 219 (4.1%) and 31 out of 219 (14%) were positive by RBPT and ELISA, respectively. All the sera samples were PCR negative. Similarly a total of 753 sera were collected various livestock species and 51/753(6.7%) and 95/753 (12.6%) were positive by RBPT & ELISA, respectively. The study clearly indicated the prevalence of brucellosis in both livestock and humans.

Clinical samples from animals and human were analyzed for *Listeria by* isolation, PCR and ELISA. Samples were collected from both man and animal sources and subjected to diagnosis of listeriosis. Out of 96 samples processed, only 2 isolates could be recovered, which could be confirmed by bacterial identification kit and genus and species specific PCR.

In a contract research project with Bangalore Milk Union Ltd, Karnataka, a total of 1624 milk samples were received from Bangalore urban and rural milk societies in the project. Milk samples were tested for anti-*Brucella* antibodies by milk ring test and 109 out of 1624 (6.71%) milk samples were positive.

The database of the Directorate has large number of data on outbreaks/cases of parasitic diseases of livestock. A detailed study on Trypanosomiasis of bovines is underway. A total of 247 cattle sera samples were collected from Odisha and were screened for the presence of antibodies against *Trypanosoma evansi* by indirect ELISA. An overall 13.25% samples were recorded as positive. Out of these samples, 0.6 % shown positive from Jajpur, 5.4% from Kendrapara, 2.4% from Puri and 4.75% from Cuttack. Among cattle, buffaloes, sheep and goats, ascariasis appeared as the highest occurring parasitic disease followed by strongylosis, fascioliasis, babesiosis, amphistomiasis, trypanosomiasis, theileriosis and so on and Trypanosomiasis caused highest death followed by amphistomiasis, fascioliasis, theileriosis, babesiosis and so on when analysed using the data of 1990-2011.

The Directorate has a unique National Livestock Serum Bank and has developed a mini software for cataloguing of the sera received. The results of the sera tested for IBR and Brucellosis are also computerized and dispatched at the earliest to the stakeholders. During the period under report, a total of 4671 serum samples of various livestock species were received from various collaborating centers of AICRP as well as from other places.

Interesting findings were revealed in Bovine mastitis project (NAIP funded). From 117 different samples, a total of 174 staphylococci were isolated and predominant were of Coagulase negative methicillin resistant Staphylococci. The most predominant *Staphylococcus* spp. were *S. sciuri* (n=61), *S. epidermidis* (n=47) followed by *S. aureus* (n=40). Quantitative multiplex PCR for detection of 14 mastitis pathogens was developed and was validated. Diverse banding patterns (A, A_1, B, C and D) were observed among the 15 *mec* positive *S. haemolyticus* isolates with type V SCC*mec* element. Majority of the milk *S. haemolyticus* isolates (66.67%, 10/15) from herd I, revealed identical band pattern A, 6.67% (1/15) of isolate from milk illustrated closely related pulsotype with two band difference from pulsotype A, 20% (3/15) of isolates from milk, udder skin and teat cup liner had a distinct pattern.Selected inflammatory mediators at RNA transcript level in milk and tissue infected with *E. coli, S. aureus* and, *Streptococcus sp.* in time course manner were evaluated.

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.

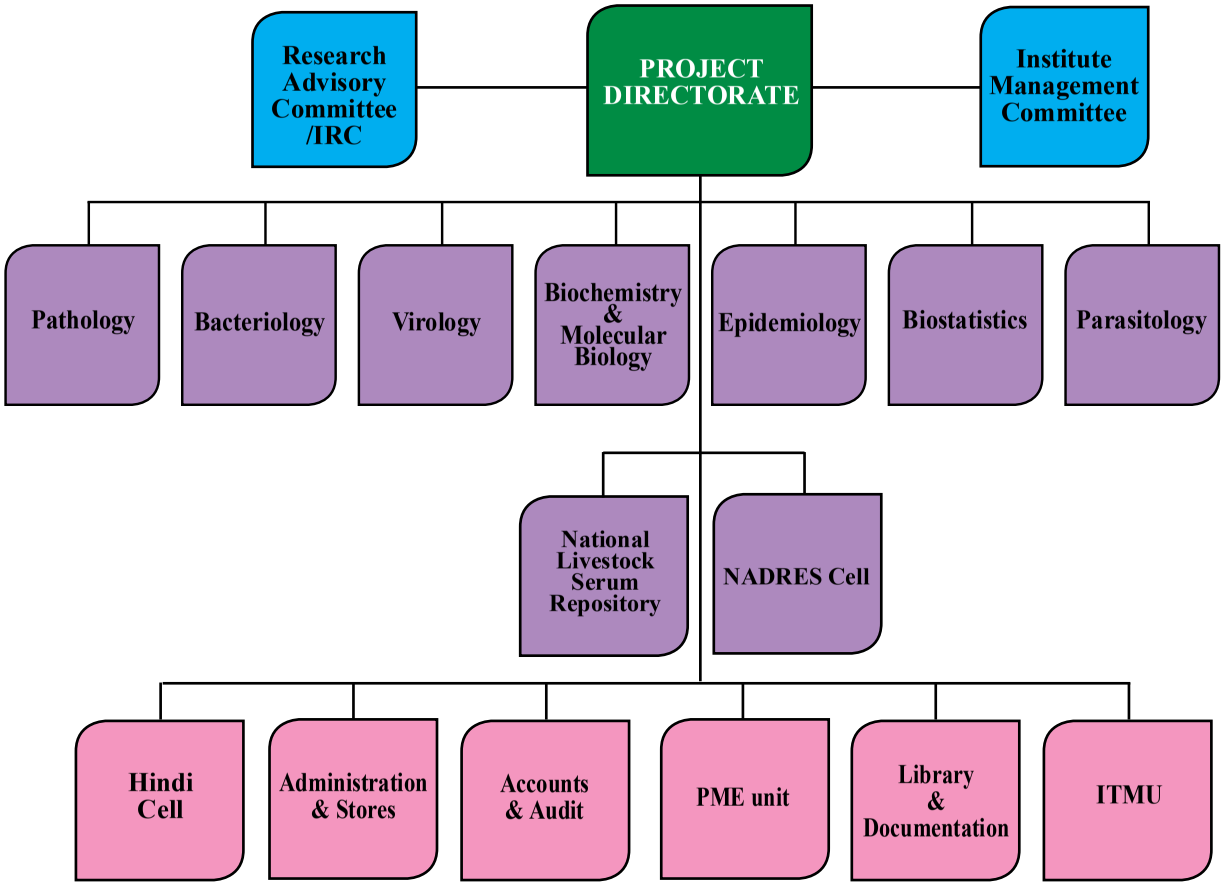
Research mandates of PD_ADMAS

- 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
Biostatistics
Parasitology
National Livestock Serum Repository
NADRES Cell
Hindi Cell
Administration & Stores
Accounts & Audit
PME unit
Library & Documentation
ITMU

Institute Research Projects

Epidemiology and forecasting of economically important livestock diseases of India

M R Gajendragad

Project Directorate on Animal Disease Monitoring and Surveillance has been compiling country wide information on livestock diseases for the last two decades. In addition to the said data, epidemiology unit of the institute has been maintaining and regularly updating various databases related to demography, livestock population, various meteorological parameters, land utility, crop production and also agro-ecological parameters. These compiled data are used for livestock disease forecasting carried out in the NADRES software.

Livestock disease profile

The livestock disease data is received from the collaborating units of AICRP on ADMAS and also from the Animal Husbandry Department of state governments. These data are entered into the disease database in the required format. As on 31st March 2012, the database has 90,104 records. Further, the data has been arranged species-wise, month-wise and district-wise. The data has been analyzed for disease ranking at national level and state level. They have also been ranked on etiological basis. The following graphs (Figs. 1, 2, 3 & 4) depict the ranking of diseases for the year 2010-11 based on etiology at national level.

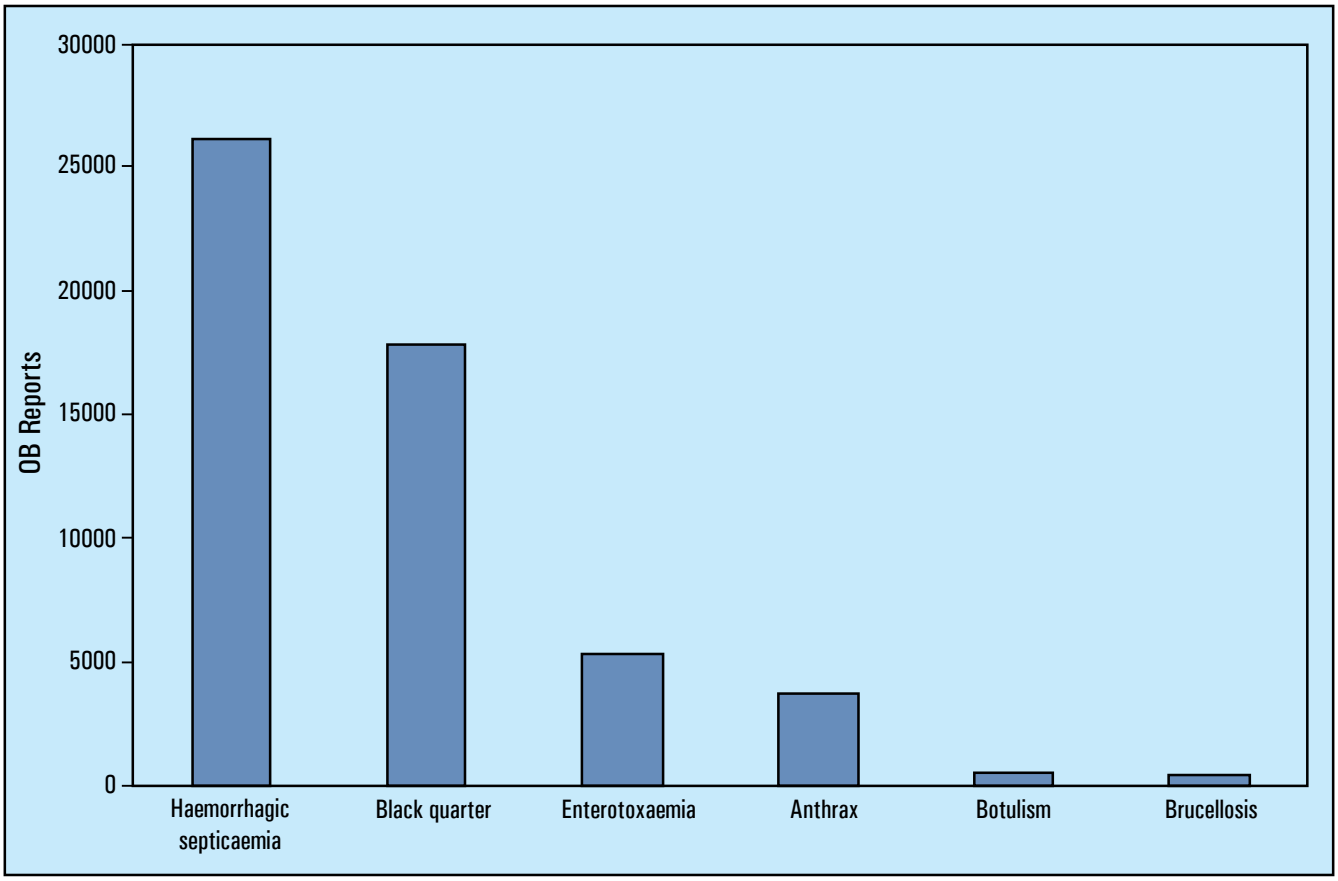


Fig. 1. Cumulative ranking of bacterial diseases.

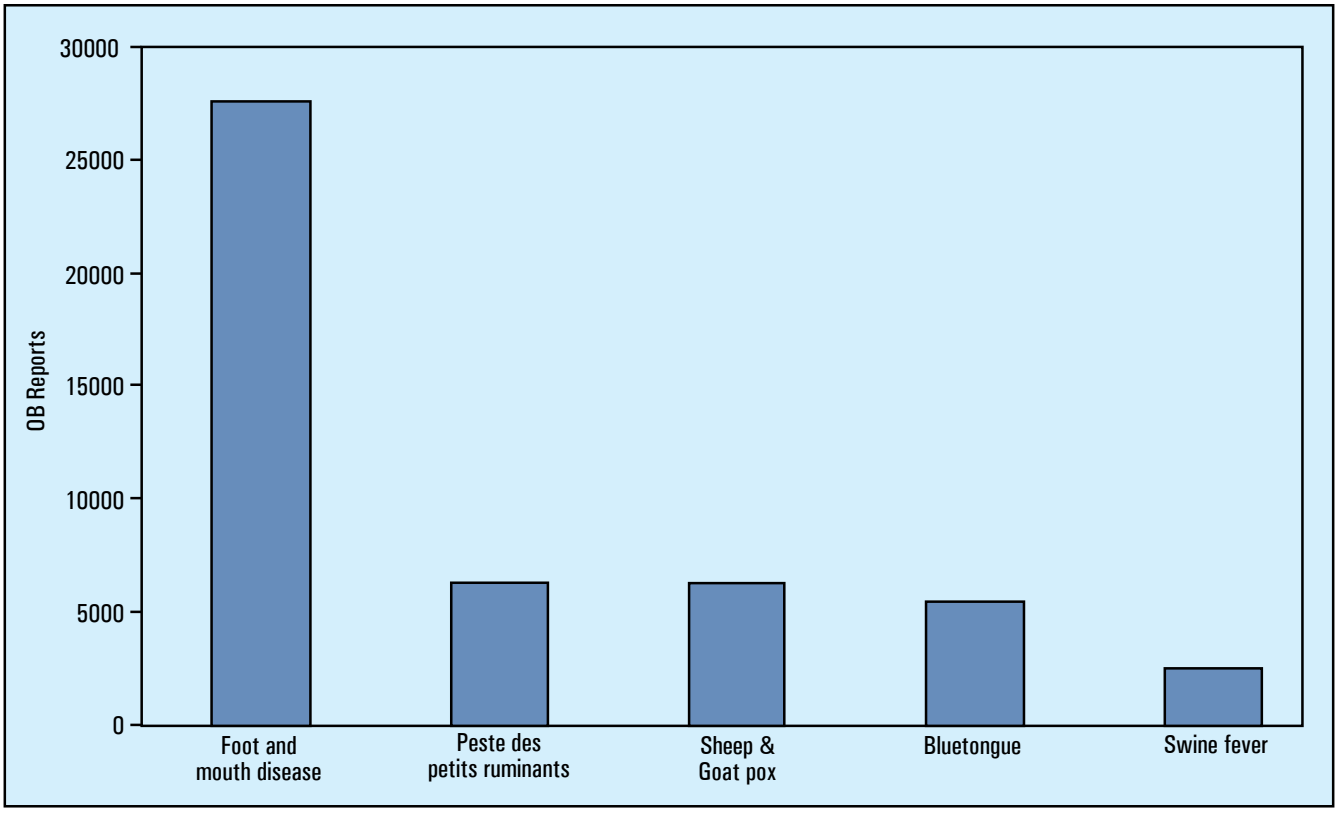


Fig. 2. Cumulative ranking of viral diseases.

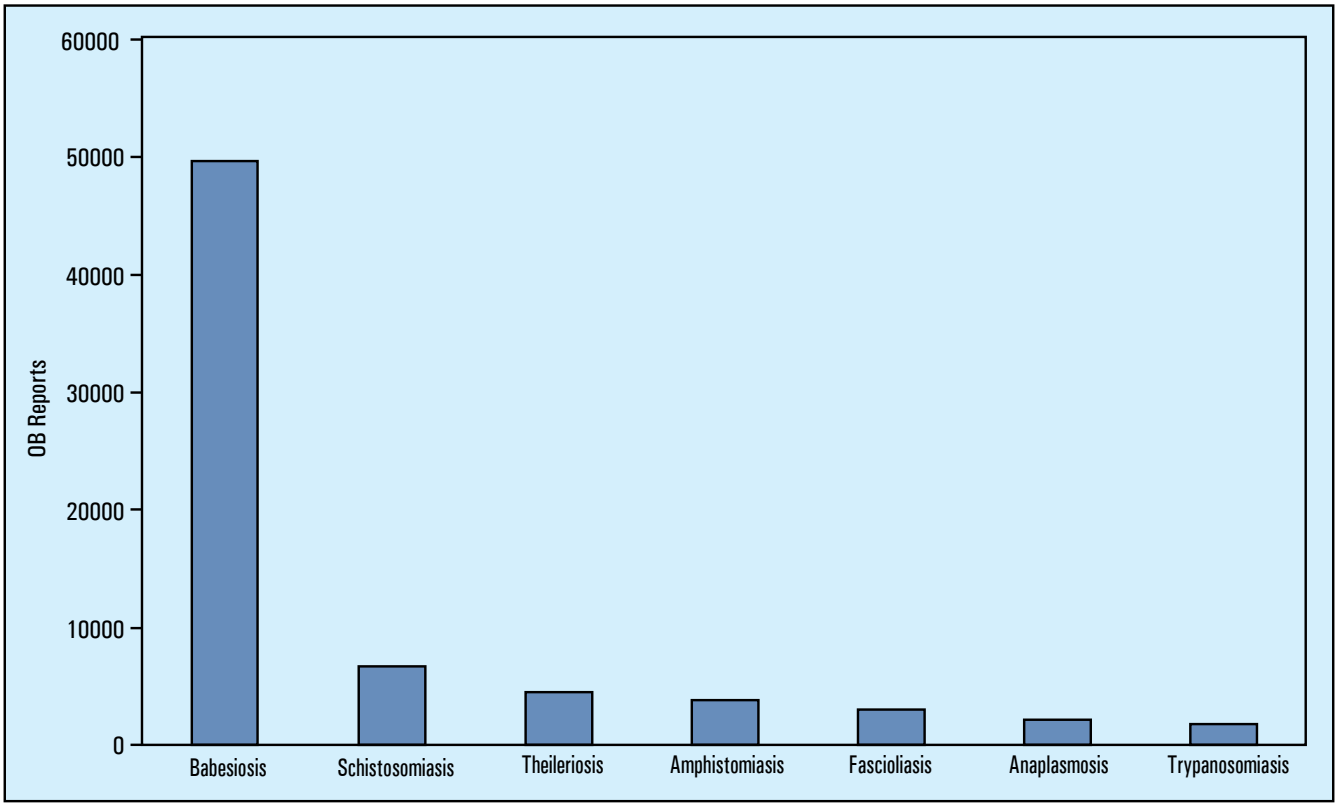


Fig. 3. Cumulative ranking of parasitic diseases.

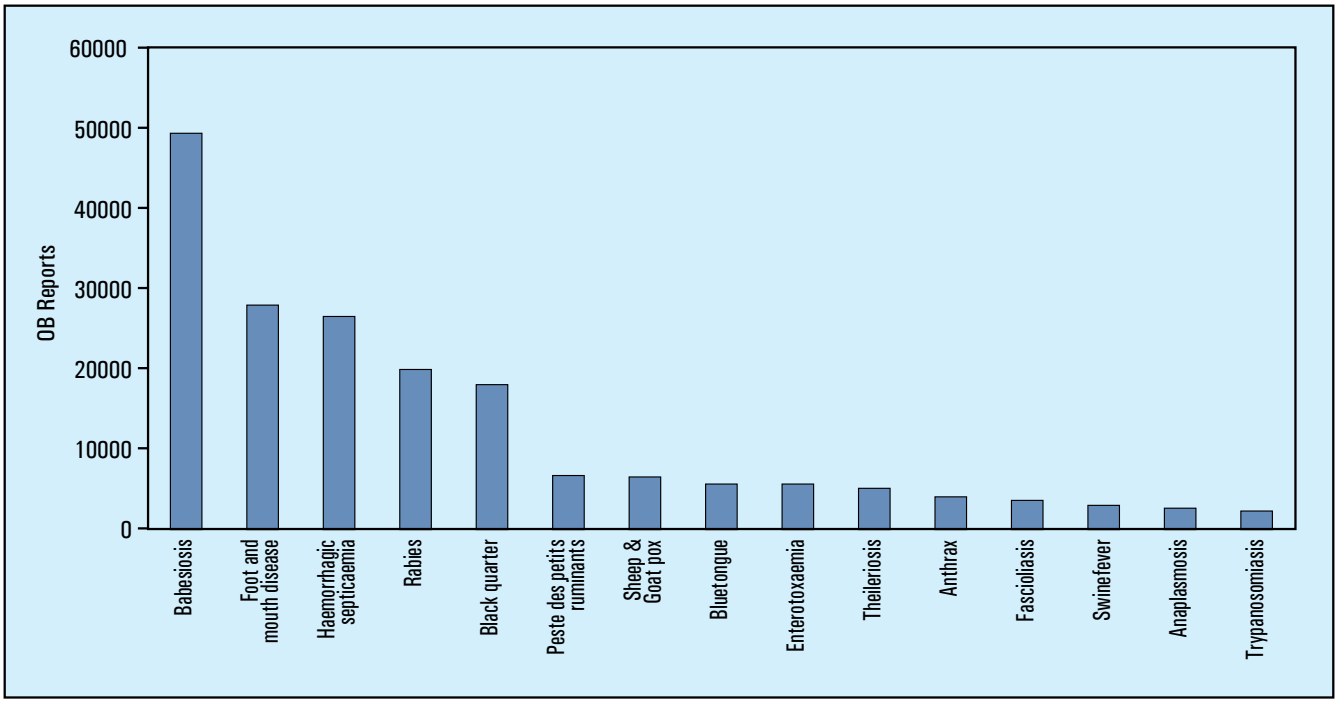


Fig. 4. Cumulative ranking of livestock diseases : a comparison.

Although the cumulative disease ranking in the country shows that HS, FMD and Babesiosis are the most reported diseases, the top spots were occupied by HS, PPR and Enterotoxemia during the year 2010 – 1 (Fig. 5). There is a clear cut decline in FMD cases, however, the other diseases viz., anthrax, sheep and goat pox, BQ are being reported consistently. Amongst the parasitic diseases, theileriosis and trypanosomiasis were recorded where as babesiosis was not recorded during 2010-11.

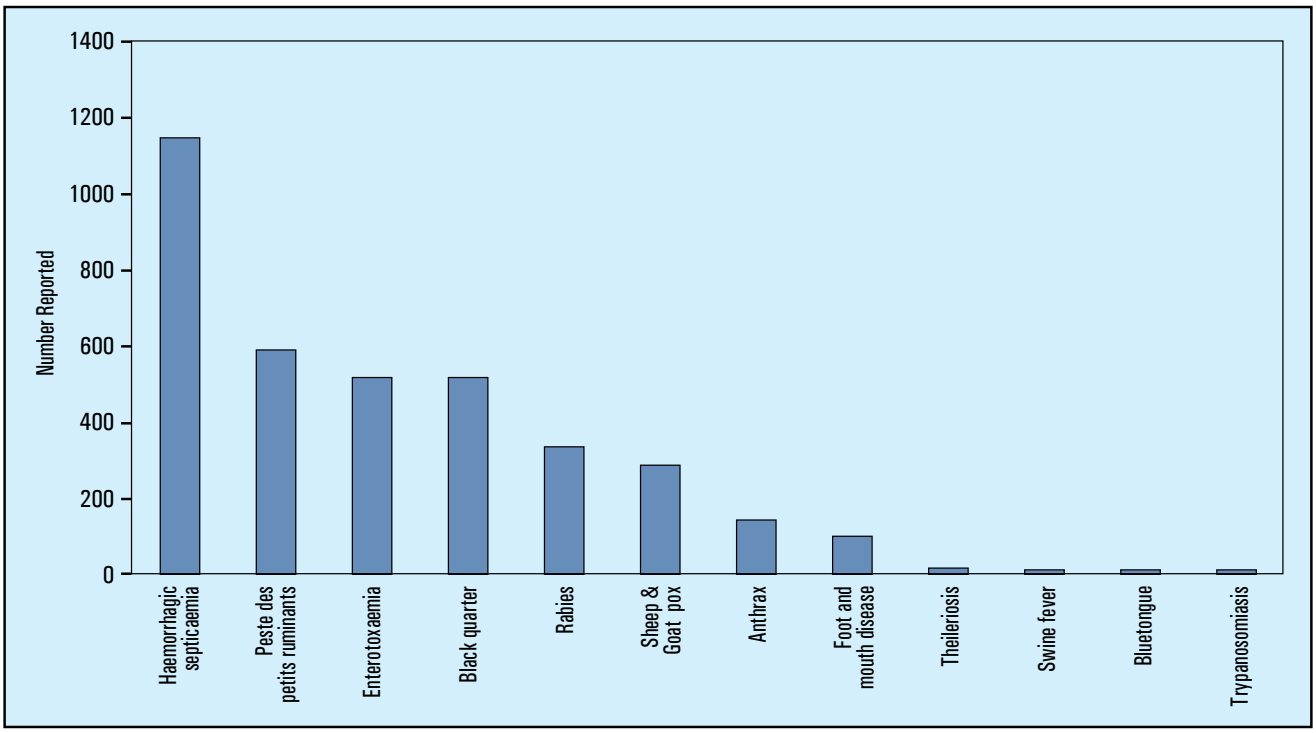


Fig. 5. Ranking of diseases during 2010-11.

Month-wise analysis

Month-wise analysis of the data shows that diseases such as Anthrax, BQ, ET, FMD, HS, PPR and Sheep & Goat pox were reported throughout the year (Fig. 6).

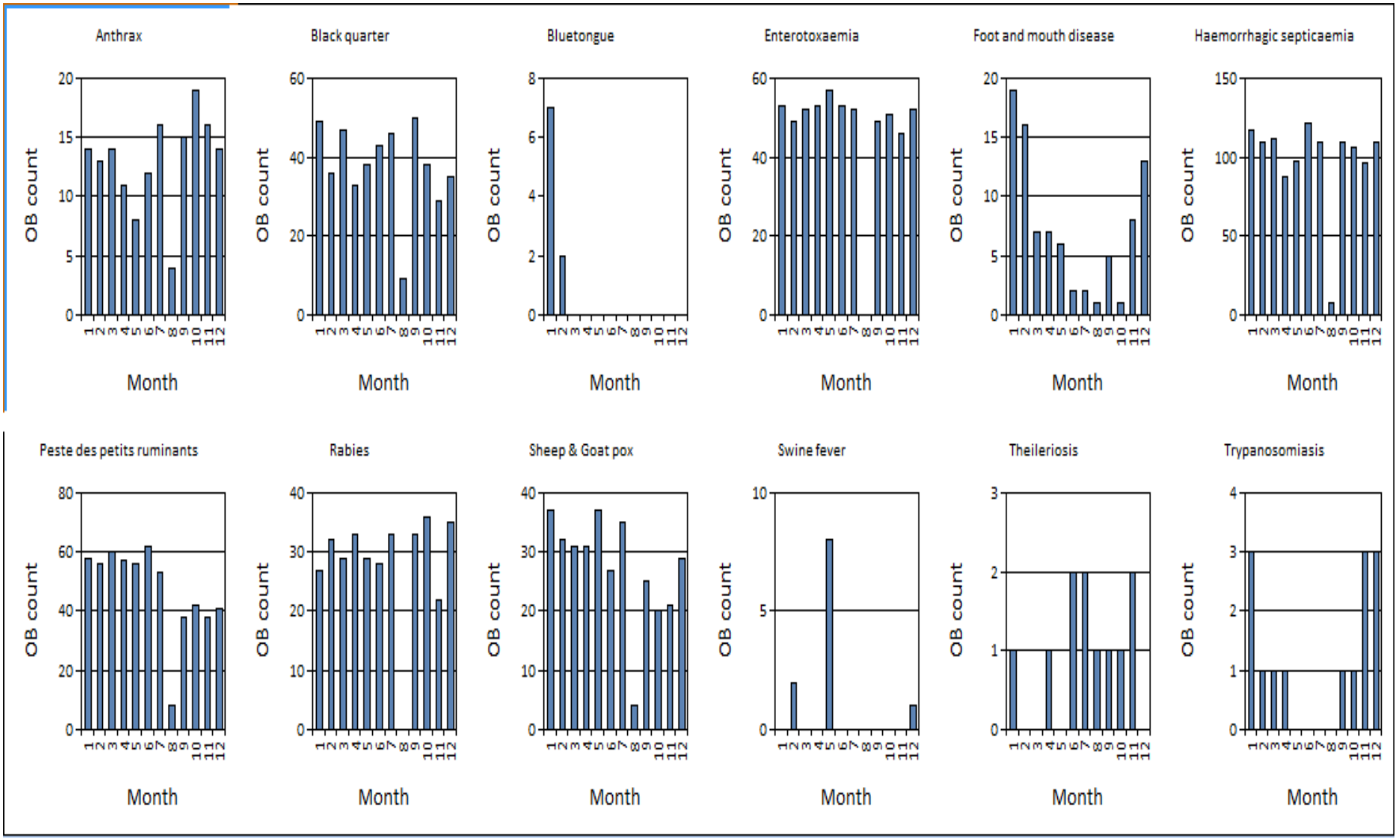


Fig. 6. Month-wise occurrence of livestock diseases during 2010-11.

Detailed analysis of livestock diseases

During the year under report two major diseases, Anthrax and Haemorrhagic septicaemia were selected for detailed analysis.

Analysis of cumulative data on Anthrax

The epidemiology of anthrax (from 1991 to 2010) has been constructed based on the disease records available in National Animal Disease Referral Expert System (NADRES). As per NADRES data, anthrax features as one of the top ten diseases reported in India and also as one of the major causes of death in livestock. Anthrax has been reported in eighteen states *viz.*, Andhra Pradesh, Assam, Bihar, Chhattisgarh, Gujarat, Himachal Pradesh, Jammu and Kashmir, Jharkhand, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Odisha, Rajasthan, Tamilnadu, and West Bengal during the last two decades (1991-2010) (Fig.7).

There has been a constant variation in the percentage of states reporting anthrax (calculated as percentage of total states reporting disease data), but an overall increase was seen since 1999 onwards, which might be due to the gradual spread of the disease. The occurrence of anthrax showed a progressive trend with a gradual increase in the number of outbreaks since 1992, with a peak seen between 2000 and 2002. From 2002 onwards, there was a gradual decline in the occurrence of anthrax (Fig. 8). This could be attributed to awareness amongst the farming community and the control measures taken up by the authorities.

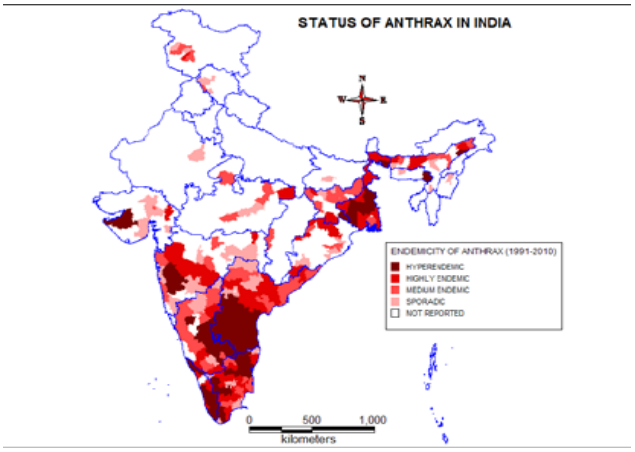


Fig. 7. District-wise status of Anthrax in India. Various shades of red indicate level of endemicity of the disease. Light red colour indicates sporadic cases while deep red indicates hyper endemic areas. White areas denote the places where the disease has not been reported.

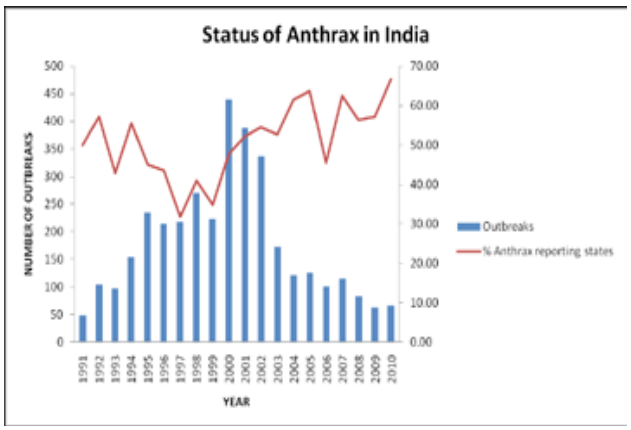


Fig. 8. Year-wise status of Anthrax with percentage of states reporting the disease.

Though the occurrence of anthrax is generally decreasing, it is still hyperendemic or hyper enzootic in many pockets of the country. This can be attributed to lack of public awareness and effective control programmes. Anthrax occurs as a persistent threat in many districts of south zone, where if control measures are relaxed, can lead to severe epidemics. Many cases of anthrax go unreported. Thus, the reported outbreaks available only provide an index of the magnitude of the disease in India and could be an underestimate of the extent of the problem. Unfortunately, the mere absence of report of outbreaks is no proof of absence of the disease.

In India, the occurrence of Anthrax varies greatly in different zones of the country as the climate and ecological factors varies greatly between the zones. Anthrax has been reported widely in the south zone of the country followed by east, west and north-east zones.

India has different agro-climatic zones and the occurrence of anthrax across the country depends on them. The very high, high, medium and low risk areas have been mapped based on the 20 years reports of anthrax occurrence. Most of the districts of Andhra Pradesh, few districts of Karnataka, Tamilnadu, Kerala, Gujarat, West Bengal and Assam have been identified as very high anthrax pathozone (Fig. 9).

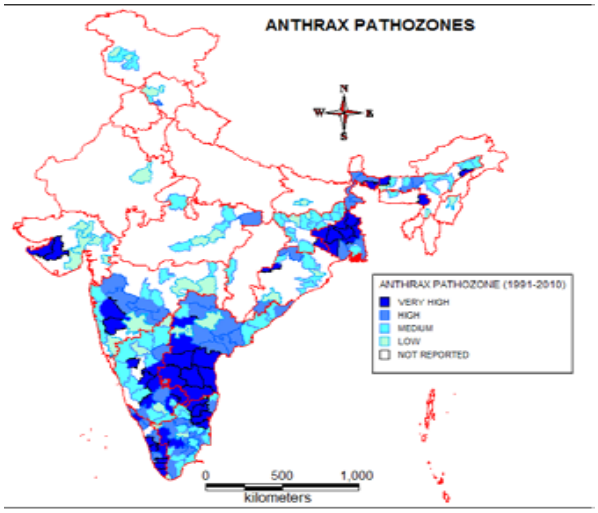


Fig. 9. Anthrax Pathozones. Light to deep blue shades indicate severity of pathozones in that order. White areas indicate the places where the disease has not been reported.

The decadal and quinquennial trend of anthrax occurrence shows that there are periodic fluctuations in its occurrence in different states. Compared to 1991-2000, there is an overall decrease in the occurrence of anthrax in 2001-2010, except in few states like Karnataka, Tamilnadu, Odisha, and Assam. On analysis of data at five year intervals between 1991 and 2010, highest occurrence was observed in the 1996-2000 period in most states except in case of Karnataka, Tamilnadu, Odisha, and Assam where, highest occurrence was seen in 2001-2005. Newer districts reporting anthrax can be viewed as a threat as it indicates spread of the disease (Fig. 10).

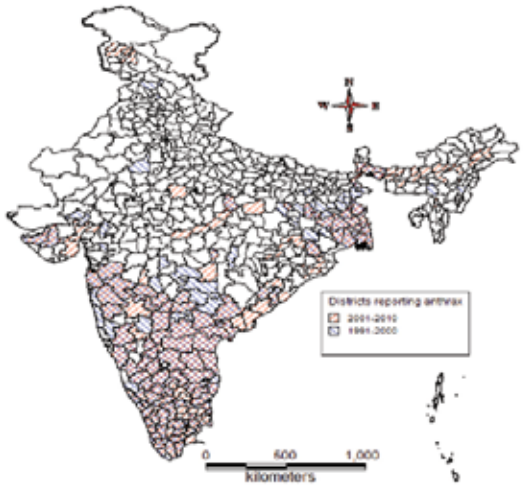


Fig. 10. Decadcal analysis of Anthrax outbreak reports.

Analysis of cumulative data on Haemorrhagic Septicaemia

The epidemiology of HS (from 1991 to 2010) has been constructed based on the disease records available in NADRES. HS features as the second most reported disease in India during the last two decades and is the cause of maximum number of deaths reported in livestock in the country. HS has been reported in twenty five states *viz.,* Andhra Pradesh, Arunachal Pradesh, Assam, Bihar, Chandigarh, Chhattisgarh, Goa, Gujarat, Haryana, Himachal Pradesh, Jammu and Kashmir, Jharkhand, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Mizoram, Odisha, Punjab, Rajasthan, Tamilnadu, Uttaranchal and West Bengal during the last two decades (1991-2010) (Fig. 11).

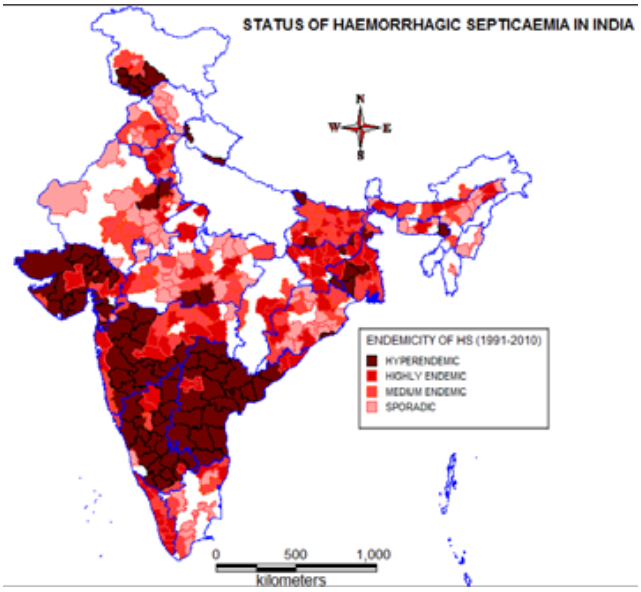


Fig. 11. Status of Haemorrhagic septicaemia in India.

There has been a constant variation in the number of states reporting HS. A progressive trend was seen in occurrence of HS since 1992. Maximum outbreaks were reported in 1998. There was a gradual decline in the number of HS outbreaks reported since 1998 (Fig. 13). This could be attributed to the vaccination strategies followed by the different state governments.

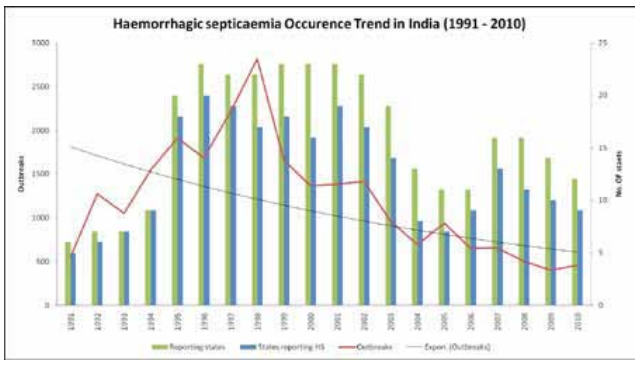


Fig. 12. Temporal analysis of HS trends in India.

The occurrence of HS has been reported in all the six zones of the country. High prevalence of HS has been reported in the south zone of the country followed by west, central, east, north and north-east zones. Though there was a lot of variation in the number of outbreaks reported across the years in the different zones, a peak was seen every three years and the trend of HS occurrence seemed to follow a similar pattern in south, west and central zone. The pattern of disease occurrence over the two decades is similar in east and north-east zones. These similarities could be due to the geography and meteorological conditions in these zones. India has different agro-climatic zones and the occurrence of the disease across the country varies greatly. Based on the 20 years' reports of HS occurrence, the very high, high, medium and low risk areas have been mapped. Most of the districts of Andhra Pradesh, Karnataka, Maharashtra, and Gujarat have been identified as very high HS pathozone belt. Among the states of south zone, Tamilnadu and Kerala have effectively contained the disease with proper vaccination and most of their districts fall under medium or low HS pathozone. There are pockets of very high HS pathozones throughout the country. In the East zone, many districts bordering the junction between the states of Bihar, Odisha and West Bengal belong to high HS pathozone (Fig. 14).

The decadal and quinquennial trend of HS occurrence are shown below (Fig. 14). Considerable variation in year to year occurrence of the disease is seen in different states. There is an overall decrease in the HS outbreak reports in the different states of the country during 2001-2010 compared to 1991-2000, except in Bihar, Odisha and Assam. On analysis of data at five year intervals between 1991 and 2010, highest occurrence was observed in the 1996- 2000 period in most states.

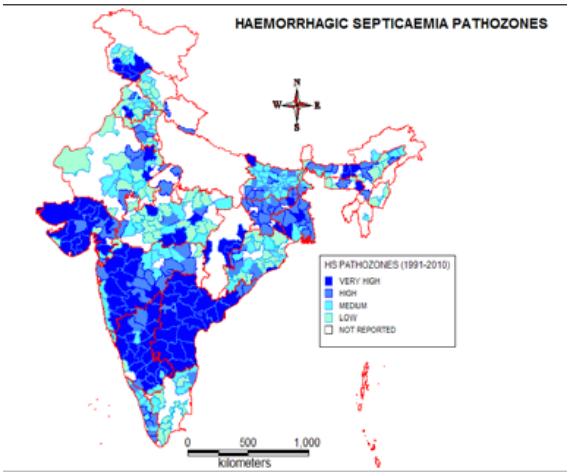


Fig. 13. HS pathozones

There has been a gradual decline in HS outbreaks since 1996-2000 and many states have reported fewer outbreaks during 2006-2010. The decline in the occurrence of HS in India can be attributed to the availability of cost-effective programmes for prevention and control. Many districts of Madhya Pradesh, Odisha and Rajasthan have reported the disease for the first time during the last 5 year study period. Newer districts reporting the disease can be viewed as a threat as it indicates spread of the disease (Fig. 14.).

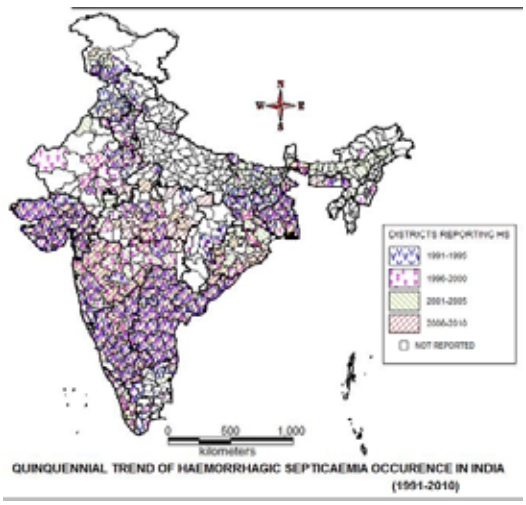


Fig. 14. Quinquennial analysis of HS outbreak reports.

Demography

For the purpose of epidemiological analysis the country has been divided into 609 districts and each village is considered as a single unit and termed "epi-unit". The new districts created after 2006 have been added in the database. However, the data pertaining to these new districts will be included from 2012 for the purpose of analysis.

Livestock population profile

The latest livestock population data has been downloaded and formatted as per the requirements for epidemiological analysis.

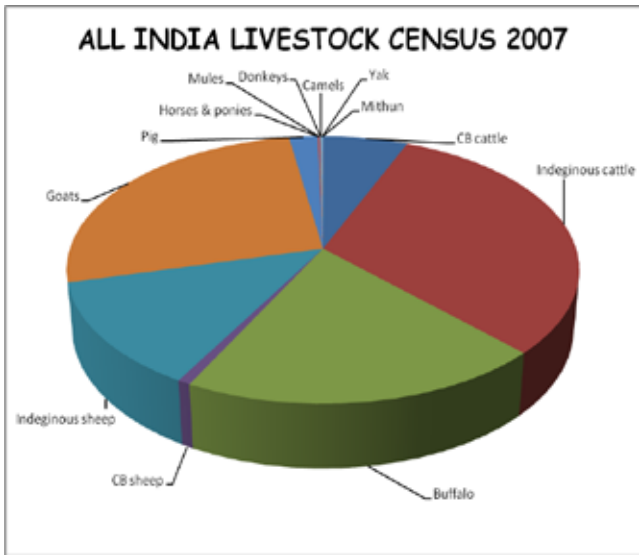


Fig. 15. All India Livestock Census 2007

Agro-ecological factors

This data has been compiled using the data of the National Bureau of Soil Survey and Land Use Planning (NBSSLUP), an ICAR institute. This data has been organized into the need of the epidemiological analysis and lined to the livestock disease profile.

Meteorological profile

The data have been collected on daily basis and at district level from 81 meteorological stations across India. These stations were grouped into 44 meteorological zones using agro-ecological zone profile. New set of data is being generated and collated.

Based on the various risk factors involved, the ecopathozones of economically important diseases were prepared. Ecopathozones for PPR (Fig. 16) and CSF (Fig. 17) are given below.

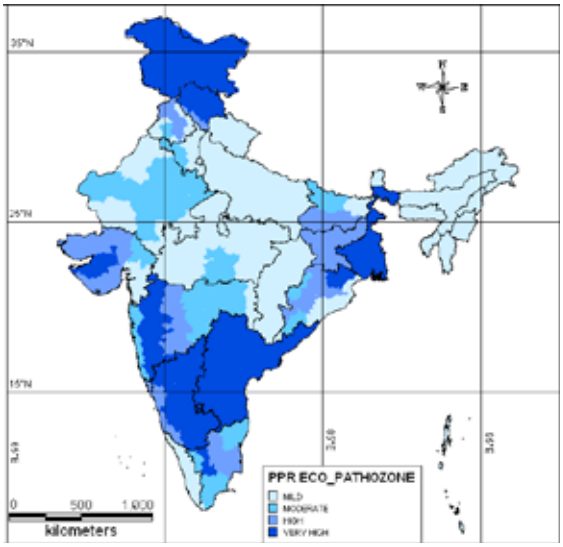


Fig. 16. PPR Ecopathozone

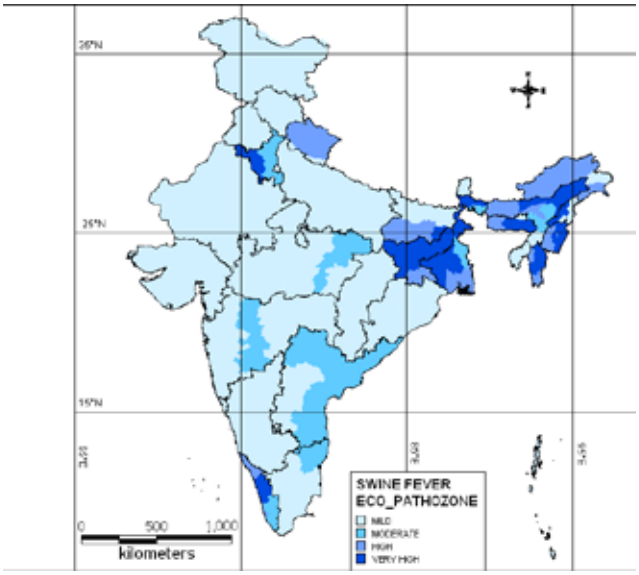


Fig. 17. CSF Ecopathozone

Animal Disease Forecasting

The latest data collected as indicated earlier has been incorporated in the model and integrated to each district for each calendar month for a specific disease. A logistic regression analysis for 15 economically important livestock diseases shown in the table below is being carried out.

Table 1. List of diseases incorporated in livestock disease forecasting model.

Bacterial diseases	Viral diseases	Parasitic diseases
Anthrax	Bluetongue	Babesiosis
Black Quarter	Classical Swine Fever	Fascioliasis
Enterotoxaemia	Foot and Mouth Disease	Theileriosis
CCPP	*Peste des petits ruminants*	Trypanosomiasis.
Haemorrhagic Septicamia	Rabies	
	Sheep & Goat Pox	

The model 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 using *MapInfo* software for all the 15 diseases. The latest forecasting maps are uploaded into the NADRES software accessible at www.nadres.res.in

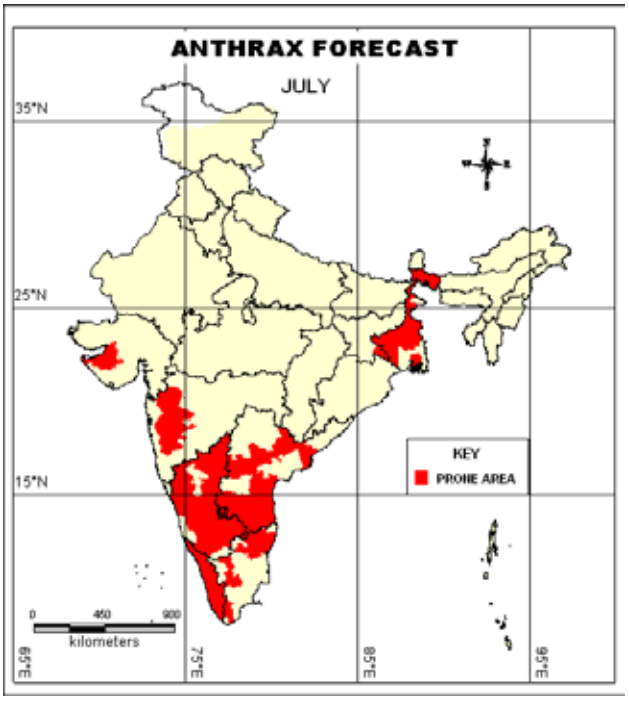


Fig. 18. Anthrax forecast map for the month of July 2012.

Epidemiological survey and estimation of economic impact of PPR in sheep and goats

V. Balamurugan, M. R. Gajendragad, B. Ganesh Kumar, G. Govindaraj and P. Krishnamoorthy

Peste des petits ruminants (PPR) is one of the highly contagious and economically important viral diseases of small ruminants, especially goats and sheep, with morbidity and mortality rates as high as 100% and 90%, respectively. India has a considerable sheep population of 78.7 million and a goat population of 140.05 million. Now, PPR is enzootic in India as outbreaks occur in small ruminants regularly throughout the country, and is a major constraint in small ruminant production incurring great economic losses in terms of morbidity, mortality, productivity losses with trade restriction. Epidemics of PPR have enormous consequences in terms of the dramatic effects this disease can bring about on livestock productivity and the high costs of control or eradication. Epidemics affect not only individual farmers but also the agricultural industry and as a consequence, the national economy. PPR is present in countries, which are either developing or under-developed thereby adding to the economic woes. An estimate in the absence of authentic data on the losses in India due to PPR is an arbitarory. Comprehensive information on epidemiology of PPR as a whole in India is not available except some few reports. Economic loss due to PPR is not quantified comprehensively so far at the national and regional or state levels. The quantification of economic losses due to any disease in animals is very important since it helps in prioritizing the research on animal health issues; and designing appropriate control programme for PPR disease in turn it helps in optimal utilization scarce resources. Hence, this project was initiated during January 2012 with objectives of evaluating the economic loss of PPR in sheep and goat in India and also to assess the impact of control programmes of PPR in sheep and goats, taking into consideration of various parameters, like variations in the price across the states, over the time periods (temporal variations) and other assumptions using standard economic models and survey procedures. Besides the secondary data, the primary date will also be collected during the PPR occurrence period to assess the direct and indirect losses due to disease. During the period under report following work has been initiated. 1. Literature on epidemiological and economic aspects of PPR has been reviewed 2. Secondary data collection on PPR outbreaks, incidence rate etc in small ruminants is in progress.3. Schedule preparation to collect primary data to assess economic losses of PPR is in progress.

Study on epidemiology of PPR in India

V. Balamurugan, P. Krishnamoorthy, M. R. Gajendragad, K. Prabhudas, H. Rahman, A. Sen, V. Bhanuprakash, K. K. Rajak and A. B. Pandey

Seroprevalence of PPR in cattle and buffalo in Southern India

Seroprevalence of *Peste des petits ruminants* (PPR) in cattle and buffaloes was carried out during the period 2009–2010 using the randomly collected serum samples from different parts of southern peninsular India. The report presents the results of PPR virus (PPRV)- specific antibodies in situations where either the subclinical or inapparent or non-lethal infection was present in cattle and buffaloes. A total of 2,548 serum samples [cattle = 1,158, buffaloes = 1,001, sheep = 303 and goat = 86] were collected and screened for PPRV antibodies by using a PPR monoclonal antibody-based competitive ELISA kit. Analysis of 2,159 serum samples indicates an overall 4.58% prevalence of PPRV antibody in cattle and buffaloes. The true prevalence of PPRV antibodies in cattle and buffaloes were 5.21 and 4.82, respectively. The percentage prevalence of PPRV antibody in Karnataka, Tamilnadu. Puducherry and Andhra Pradesh were 4.23, 9.65, 4.32 and 1.3, respectively in bovines. The PI

values obtained in c-ELISA from tested bovine samples were depicted in a graph (Figs. 1A and B), in which more number of suspicious cases (n=47) fell between 25 and 40 PI values, as the cutoff value taken was based on the sheep and goat samples described earlier. In the distribution of the PPRV-specific antibodies in positive cattle and buffaloes, 80 base level positive samples were having the PI range from 40 to 60, nine weak positive samples with PI values of 60 to 80 and ten strong positive samples had a range from 80 to 90. There was no significant difference between the cattle and buffaloes in the percentage positivity for PPRV antibodies.

Serum samples from different states of the country through coordinating units of PD-ADMAS, submitted to the Serum bank of the PD_ADMAS, Bangalore were also subjected to screening of PPRV antibodies by using the PPR-competitive ELISA kit. The overall details of the results are presented in the Table. 1 and percent positivity in graph (Fig.2).

Seroprevalence/clinical prevalence of PPR in suspected livestock species in India

In IVRI, Mukteswar, serum and clinical samples from different geographical locations of the India obtained through various organizations including organized goat/sheep fa rms, the field, 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 competitive ELISA kit and the PPRV antigen in the infected swab materials/post-mortem samples was detected by using PPR Sandwich ELISA kit. A total of 1070 serum samples (Goat-570; Sheep-421; Bovine/others-79) were screened for PPRV antibodies by using PPR C-ELISA kit, the percentage positivity 46.5, 54.2 and 26.6 were observed in goat, sheep, and bovine respectively (**Table 2).** A total of 529 clinical samples (Goat-378; Sheep-148; bovine/others-3) were screened for PPRV antigen by using PPR S-ELISA kit, the percentage positivity 31.48 and 44.6 were observed in goat and sheep, respectively (Table 3).

Conclusions

- Based on screening of 2159 serum samples, 4.58 % prevalence of PPR antibodies in cattle and Buffaloes in Southern Peninsular India was observed
- The prevalence rate of PPRV antibodies 10.52 % was observed in livestock species based on screening of random 4787 serum samples.
- The prevalence rate of PPRV antibodies 48.04 % was observed in livestock species based on screening of 1070 serum samples from suspected cases of disease.

In overall, the present study describes sero-surveillance of *Peste des petits ruminants* (PPR) in livestock carried out during the period report using the serum samples randomly collected from different parts of India. In general, the percent 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. The percent 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 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. A small proportion of cattle and buffaloes do have antibodies to this PPR virus, as per the other published findings from other countries. However, the fact that the implications of these antibodies

are unknown at this stage and that further work may be required to understand their significance. Hence, 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.

Table 1. Cumulative results of randomly collected serum samples screened for PPRV antibodies

State	Cattle	Positive	Buffalo	Positive	Sheep	Positive	Goat	Positive	Total	Positive	Over all % positive
Andhra Pradesh	298	10	60	8	58	44	64	36	480	98	**20.42**
Karnataka	350	8	1001	44	303	69	86	8	1740	129	**7.41**
Puducherry	440	19	-	-	-	-	-	-	440	19	**4.32**
TamilNadu	259	25	-	-	-	-	18	2	277	27	**9.75**
Manipur	332	-	21	-	31	-	223	3	607	3	**0.49**
Jammu and Kashmir	447	33	-	-	-	-	-	-	447	33	**7.38**
Rajasthan	48	10	52	16	34	13	50	6	184	45	**24.46**
Maharashtra	105	32	75	29	25	10	82	32	287	103	**35.89**
Gujarat	167	5	59	9	28	15	71	18	325	47	**14.46**
Total	**2446**	**142**	**1268**	**106**	**479**	**151**	**594**	**105**	**4787**	**504**	**10.53**

Table. 2 Seroprevalence of PPR in suspected cases from livestock species in India

	Serum samples tested			Serum samples Positive			Percent (%) Positivity		
Place , District , State	**O**	**C**	**B**	**O**	**C**	**B**	**O**	**C**	**B**
Hissar, Haryana	167	55	20	129	25	2	77.3	45.5	10.0
CADRAD* IVRI,UP	25	67	58	2	10	19	8	14.9	32.8
Kartholi, Godhwal, J&K	22	62	--	4	43	--	18.18	69.3	-
Nammakal, Tamilnadu	-	4	--	--	--	--	-	-	-
Guwahati, Assam	-	50	--	--	33	--	-	66.6	-
Ajmer, Rajasthan	44	50	--	20	36	--	45.5	72.0	-
Parbhani, Maharashtra	-	184	--	--	66	--	-	35.9	-
Bareilly, UP	92	45	--	59	26	--	64.1	57.8	-
Palampur, HP	1	9	--	--	3	--	-	33.3	-
Agartalla, Tripura	--	7	--	--	1	--	--	14.3	-
Gujarat	70	--	--	14	--	--	20	--	-
Shitla and Nathuakhan Almora, Uttarakhand	--	21	1	--	15	--	--	71.43	-
Patna, Bihar	--	16	--	--	7	--			
Sub Total	**421**	**570**	**79**	**228**	**265**	**21**	**54.2**	**46.5**	**26.6**
Total	**1070**			**514**			**48.04**		

*Samples were received from CADRAD (*83 samples received, 50 were positive and 23 were negative), that did not have a suitable code. O-Ovine-Sheep; C-Caprine-Goat; B-Bovine-cattle.

Table. 3. Clinical prevalence of PPR in suspected cases from livestock species in India

Place , District , State	Samples tested			Samples Positive			Percent (%) positivity		
	O	C	B/o	O	C	B/o	O	C	B/o
Hissar, Haryana	112	36	-	52	22	-	46.4	61.1	-
CADRAD*, IVRI, UP	16	133	-	14	56	-	87.5	42.1	-
Pune, Maharasthra	-	150	-	-	32	-	-	21.3	-
Kartholi, J&K	20	2	-	-	-	-	-	-	-
Tamil Nadu	-	6	-	-	-	-	-	-	-
Palampur, HP	-	08	-	-	-	-	-	-	-
Mathura, UP	-	10	-	-	1	-	-	10.0	-
Agartalla, Tripura	-	06	-	-	1	-	-	16.66	-
Guwahati, Assam	-	03	-	-	-	-	-	-	-
Gujarat	-	-	3#	-	-	-	-	-	-
Almora, Shitla & Nathuakhan Uttarakhand	-	32	-	-	7	-	-	21.9	-
Patna, Bihar	-	2	-	-	-				
Sub Total	**148**	**378**	**3**	**66**	**119**	**-**	**44.6**	**31.48**	**-**
Total	**529**			**185**			**34.97**		

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

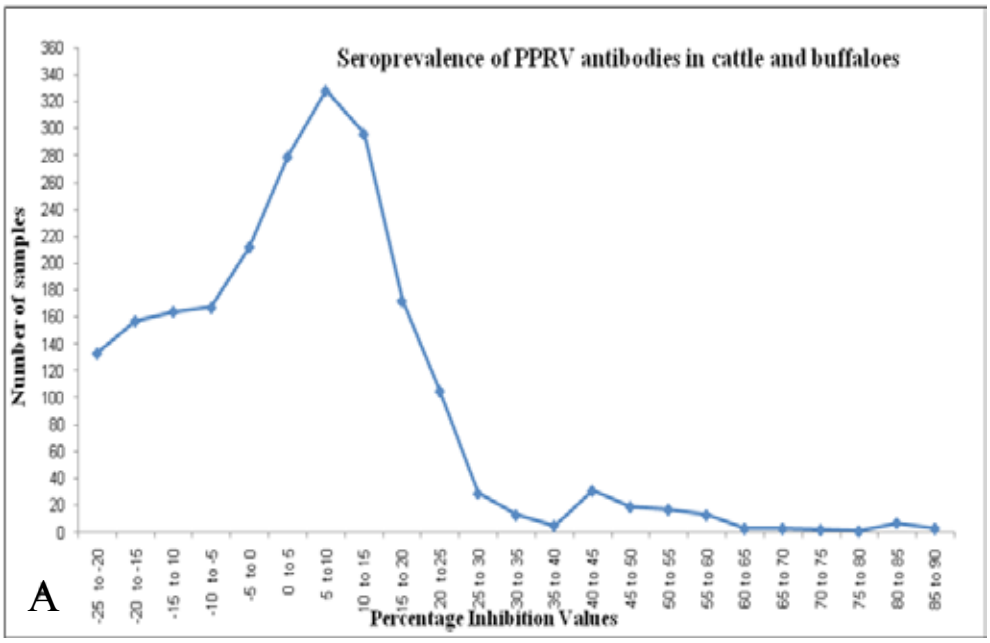


Fig. 1. Distribution of PPR Virus antibodies in cattle and Buffalo

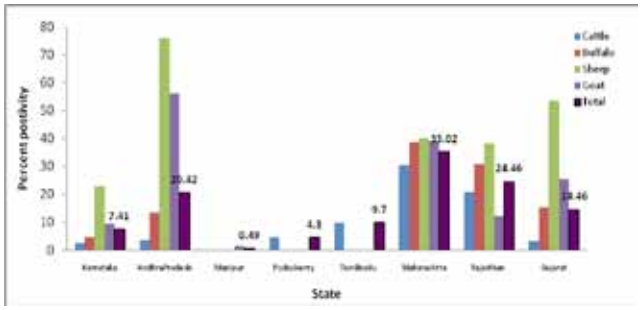


Fig. 2. Cumulative percent positivity of PPRV antibodies in livestock species in different states

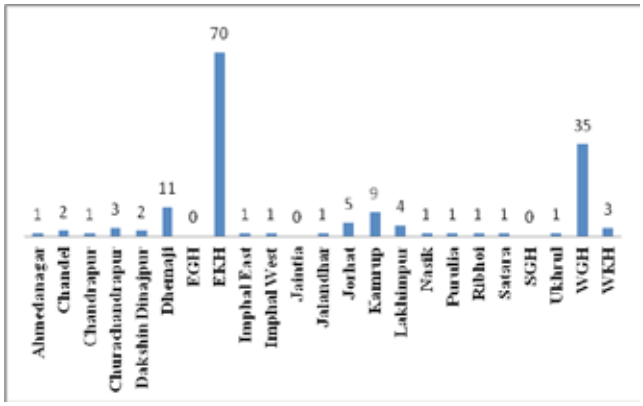


Fig. 1. District wise details of outbreaks

Epidemiology of classical swine fever in India

S. S. Patil, D. Hemadri, M. R. Gajendragad and H. Rahman

Classical swine fever (CSF) is one of the economically important diseases of pigs in India. Unfortunately, this disease has never got the attention it deserved due to the fact that major chunk of pig husbandry is mainly restricted to socially backward classes of the Indian society and also to the fact that major portion of the pig population is present in North Eastern states. Current decreasing trend in pig population [11.1 million as per 2007 livestock census as compared to 13.5 million in the previous census (2003)] may be attributed losses incurred due to infectious diseases and lack of proper/rapid diagnostics, shortage of quality vaccines etc by the aspiring pig farmers. Keeping theses points in view, epidemiology of CSF was undertaken to understand the said disease situation in the country.

CSF outbreaks in India

A total of 154 CSF outbreaks were reported during the year 2011-12. North Eastern states accounted for most of the outbreaks with the state of Meghalaya being worst hit followed by Assam. Large number of outbreaks reported from East Khasi Hills (70 nos) and West Garo Hills (35) were probably due to non vaccination of the pigs for the past two years and recent introduction of some pigs from unknown sources. District wise details of CSF outbreaks are given in the Figs. 1 & 2.

Fig . 2. CSF outbreaks during 2011-12.

Investigation of suspected CSF outbreaks in seven pig farms located in Mandya District, Karnataka.

Suspected CSF outbreaks in seven pig farms located in Mandya district were investigated during the year 2011-12. All the pig farms investigated were located within 30 sq km area. Upon enquiry, farm owners told that there are 14 private pig farms (both small and big) located in and around Mandya district and pork consumption is very high in these areas. All farm owners are dependent on cheapest source of supply of pigs and procure from private holdings (not from authorized source) located either in the borders of TN or Kerala. Most of the time the health status of these pigs are unknown to the buyers and the farm owners/pigs

are not traceable after sometimes. Swill feeding is practiced in most of these farms and freely procured garbage either from canteens of reputed private companies or hotels is fed to these pigs. Many a times Pig farmers exchange the garbage or the same vehicle keeps supplying to different farms. Besides farmers share the piglets amongst themselves and frequently keep visiting the farms for a friendly chat. Although CSF outbreaks were suspected in all seven pig farms, the disease had died out in most of the farms at the time of visit. In only one farm clinical signs resembling CSF were noticed, never the less serum samples were collected from all the pig farms and subjected to antibody screening by ELISA. Of the 21 serum samples screened, 16 were found positive. The movement route of pigs within Karnataka, Kerala and Tamilnadu is given below (Fig. 3).

Fig. 3. Movement route of pigs within Karnataka, Kerala and Tamilnadu states.

Sero-epidemiology of CSF

A total of 426 pig serum samples from Andhra Pradesh (9), Karnataka (21), Odisha (5), Manipur (64), Maharashtra (22), Madhya Pradesh (254) and Rajasthan (51) were screened for the presence of antibodies against CSFV infection using competitive ELISA. All the pigs under this study were unvaccinated. A total of 191 sera samples were positive for CSF antibodies as shown in Table 1. District wise and state wise sero prevalence of CSF is shown in Figs. 4 and 5. Cumulative and district wise sero-prevalence of CSF in India during 2010-12 is shown in Table 2 and Fig. 6.

Table 1. State wise sero-prevalence of CSF during 2011-12

S. No.	State	No. Tested	No. Positive	Percent Positive
1	Andhra Pradesh	9	0	0
2	Karnataka	21	16	76.19
3	Odisha	5	0	0
4	Manipur	64	22	34.37
5	Maharashtra	22	10	50
6	Madhya Pradesh	254	111	43.7
7	Rajasthan	51	32	62.74
Total		**426**	**191**	**44.83**

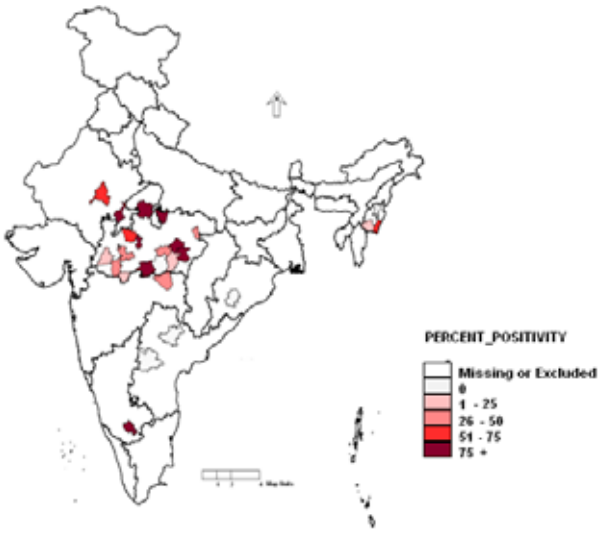


Fig. 4. District wise seroprevalence (percent positivity) of CSF during 2011-12.

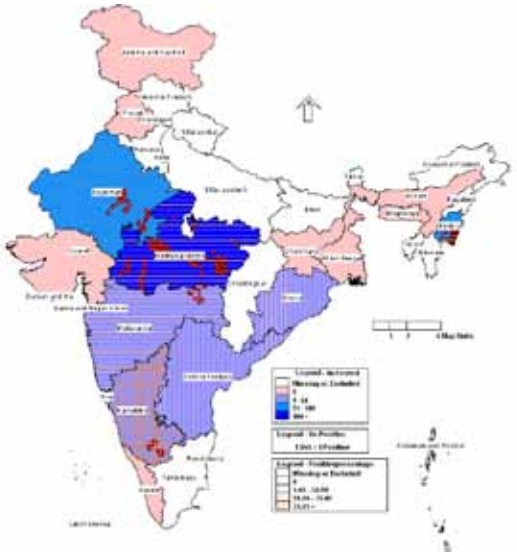


Fig. 5. State wise seroprevalence of CSF during 2011-12.

Table 2. Cumulative sero-prevalence of CSF in India during 2010-12.

S. No.	Year	No. Tested	No. Positive	Percent Positive
1	2010-11	1257	237	18.85
2	2011-12	426	191	44.83
Total		**1683**	**428**	**25.43**

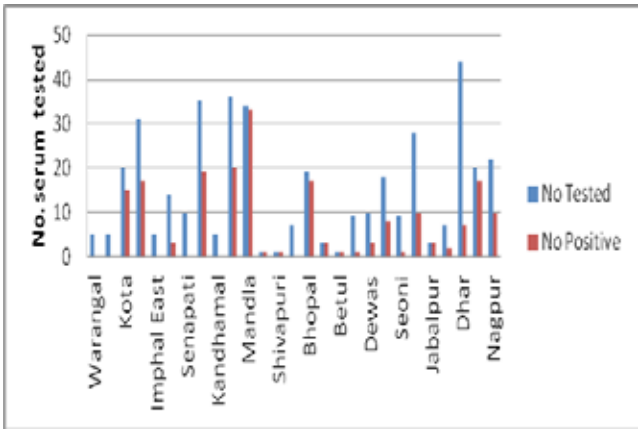


Fig. 6. District wise sero-prevalence of CSF during 2011-12.

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

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

It is essential to identify and characterize the circulating *Leptospira* isolates in various animal species and humans from different geographical locations in order to monitor the spread and zoonotic potential of these organisms. Hence this project was undertaken with an objective to investigate or monitor the leptospirosis in humans and animals using established antigen detection methods. Briefly, the isolates collected during different periods of time were characterized at the species levels using *rpoB gene* based sequence and phylogenetic analysis. The approved project which was started during November 2009 and completed October 2011.

Isolation, identification and characterization of *Leptospira* isolates

Differentiation of *Leptospira* from other spirochetes is essential to determine the true involvement of the former in the suspected cases. Therefore in this study, characterization of the isolated organisms as *Leptospira* was carried out. Briefly, genomic DNA was extracted from the cultures using QIAmp DNA mini kit. Initially, *Leptospira* genus-specific PCR was carried out to differentiate the *Leptospira* from other spirochetes. Then, 16s rRNA based PCR using E1 and E2 primers, which would amplify the 571 bp amplicon from pathogenic *Leptospira* was performed. The *secY* (translocase gene)-based PCR, which would amplify the 285bp product from pathogenic *Leptospira spp.* except *L. kirschneri species* was also employed using the G1 and G2 primer set. Further, isolates were characterized by *rpoB* gene-specific PCR, which was carried out using reported primers to amplify the partial gene sequences (≈600 bp product) for species identification. The *rpoB* PCR amplicons were purified and cloned into pGEM-T Easy vector. The recombinant plasmid DNA was isolated and insertion of PCR product was confirmed by PCR and restriction endonuclease analysis. The nucleotide sequencing of insert was carried out commercially and sequence analysis was performed with published *rpoB* gene sequences of other *Leptospira spp.* by using NCBI BLAST. Phylogenetic tree was constructed based on partial nucleotide sequences of *rpoB* gene by using Molecular Evolutionary Genetics Analysis (MEGA) version 4 program. The results of the characterization study are given below.

On analysis of 191 culture isolates, 99 strains were found positive in *Leptospira* genus specific PCR assay. Further, 52 strains were identified as pathogenic, when tested by various PCR techniques. *rpoB* gene-specific amplicons from pathogenic strains were cloned, and nt sequenced. The characterized *Leptospira* isolates were from various hosts (Human-10; Cattle-21; Buffalo-4;

Goat-5; Rat-7; Elephant-1; Dog-4). The partial *rpoB* gene sequences of the characterized *Leptospira* isolates were obtained after editing the primer sequences and submitted to the GenBank database (HM046989 to HM046997; JN388615 to JN388667).

Phylogenetic analysis (Figure not shown) of 52 isolates based on *rpoB* gene sequences revealed that 31 isolates belong to either *L. borgpetersenii* or *L. interrogans* species, 13 isolates belong to *L. inadai* subgroup or subspecies and 8 isolates belong to *L. kirschneri* species. The overall observed prevalence of *Leptospira* species viz. *L. borgpetersenii/L. interrogans, L. inadai and L. krischneri* in animals and human are 59.62%, 25% and 15.38%, respectively. Similarly, among the bovine population, the percentage prevalence of aforesaid species was 60%, 32% and 8%, respectively. In Karnataka, the prevalence rate of these species was 62.25%, 25% and 12.5%, respectively. However, true prevalence of 31.3, 13.13 and 8 percent was observed for *L. borgpetersenii /L. interrogans*, *L. kirschneri*, and *L. inadai* species subgroup, respectively in animals and humans based on analysis of total positive (n=99) leptospira cultures. On sequence analysis, in general, isolates belonging to either *L. borgpetersenii* or *L. interrogans* species showed 99 to 100 % identity with reported sequences. Similarly, isolates belonging to *L. kirschneri* species had 98-99 % identity with reported sequences. However isolates belonging to *L. inadai species* showed only 75 to 78 % identity with reported sequences of *L. inadai* species.

Monitoring of zoonotic potential of leptospirosis

As the project aimed at providing a clear epidemiological picture of leptospirosis in livestock and the zoonotic potential of leptospirosis in India, monitoring of the leptospirosis burden in livestock and human was carried out to know the prevalence of *Leptospira* species using the suspected clinical samples as well as randomly collected samples both in animals and human. Monitoring of leptospirosis was carried out from time to time using samples collected by our team and also using samples received at PD_ ADMAS for diagnosis. During the period 2009-2012, a total of 959 clinical samples (Table 1) such as blood, serum, urine, which were either collected by our team from an organized farm or received for providing diagnosis of disease, were initially subjected to isolation in EMJH medium and observed periodically for the presence of the organisms by DFM as spirochetes. Human samples were directly subjected to DFM examination for initial identification. The cultures underwent a minimum of five screenings up to 45 days to rule out as negative for *Leptospira.* Some of the samples were isolated in the EMJH medium for further characterization at species level and are at various stages of screening. In addition to isolation in EMJH medium, DNA was extracted directly from all these clinical samples and standardized PCR techniques were applied for initial identification for providing diagnosis. Out of 674 samples tested, 183 were found to be positive for *Leptospira* nucleic acid by PCR (Table 2).

Most of the *Leptospira*-PCR positive human samples had the history of pyrexia of unknown origin (PUO) and most of the persons from whom the samples were collected were veterinarians. All the positive human samples were found pathogenic and further subjected to *rpoB* gene based sequence analysis for identification of the *Leptospira* species. The salient observations in the project are as follows;

- *rpoB* gene based phylogenetic analysis identified the prevalence of *Leptospira inadai* subspecies in animals and humans in India.
- Prevalence of *Leptospira* Species namely *L. borgpetersenii ; L. interrogans ; L. krischneri* and *L. inadai* sub species was observed in India based on *rpoB* gene based phylogenetic analysis of isolated and available isolates of leptospira

- In clinical and abortion cases of the livestock-most of the Leptospira species belonged to *L. borgpetersenii* and-*L. interrogans*
- In non-clinical cases most of the *Leptospira* species were *L. inadai* subgroup or subspecies

Based on the present study, the major circulating pathogenic species of *Leptospira* in animals and human were *L. borgpetersenii*, *L. interrogans, L. kirschneri*, *and L. inadai* subgroup. Further study is required to determine the exact serovars or new species of L. *inadai.* To establish the possible seroprevalence of this species in the population, the inclusion of *L. inadai* in the battery of leptospiral antigens used for sero-epidemiological studies in MAT is recommended. It is very interesting to know the revalence *of Leptospira inadai* species in India, which till today, treated as an intermediate group in the genus. This study will surely emphasize the importance of consideration of *L. inadai* in further studies in India. However, further systematic random screening of the samples from different animals and risk personnel is required to know the prevalence rate in particular geographical locality, which again depends on the various epidemiological factors influencing leptospirosis occurrence and spread namely contaminated environments, such as water and soil, dehydration, temperatures, rainy seasons etc., This could be useful in selection of panel of antigens to be used in the MAT at different geographical location in animals and human to the extent possible. Further, abortions due to leptospirosis in bovine is common, but most times over looked. Since leptospirosis is a preventable disease, early detection is beneficial in minimizing dairy losses. Further, abortion in cattle is a major problem of the dairy industry. A majority of abortions in cattle is attributed to infectious causes. Yet, leptospirosis as an etiology of abortions is overlooked. So, monitoring of the *Leptospira* abortion in clinical case of cattle is also of importance.

Table 1. Samples from man and animals analyzed by isolation in EMJH medium for *Leptospira like organisms.*

S. No	Species	Samples tested	Samples Positive	Percent positivity
1	Cattle	515	61	11.8
2	Buffaloes	41	9	22.0
3	Sheep	49	10	20.0
4	Goats	39	8	20.0
5	Canine	74	22	29.7
6	Pigs	30	3	10.0
7	Rat	18	16	88.8
8	Horse	42	0	0.00
9	Human	151	14	9.20
Total		**959**	**143**	**15.0**

Table 2. Samples from animals and human analyzed for *Leptospira* by PCR.

S.No	Species	Samples tested	Samples Positive	Percent positivity
1	Cattle	255	65	25.4
2	Buffaloes	6	2	33.0
3	Sheep	33	0	0.00
4	Goats	29	0	0.00
5	Canine	66	18	27.3
6	Pigs	36	22	61.1
7	Rat	15	8	53.3
8	Horse	38	9	23.7
9	Human	196	59	30.1
Total		**674**	**183**	**27.0**

Epidemiology of bovine leptospirosis by using recombinant antigen based assays

M. Nagalingam, V. Balamurugan, Rajeswari Shome and H. Rahman.

Leptospirosis is a potentially fatal zoonotic transmissible disease of animals and humans caused by infection with any of the pathogenic members of the genus *Leptospira*. In bovine leptospirosis, a cow can abort as early as the fourth month of gestation but abortion after seven months is more common. *Leptospira* causes persistent infection of the kidneys leading to the excretion of *Leptospira* in the urine for months. The main economic consequences of bovine leptospirosis are due to direct or indirect losses incurred from abortion, stillbirth, infertility, failure to thrive, loss of milk production and death.

Laboratory diagnosis of leptospirosis can be complex. Serological testing is the most widely used means for diagnosing leptospirosis, and the microscopic agglutination test (MAT) is the standard serological test. Leptospirosis is frequently under diagnosed because of the nonspecific symptoms and the difficulty of performing both culture and the reference serological tests. Hence it is necessary to develop a cost-effective, safe, and efficacious diagnostic test that combines sensitivity, specificity, and laboratory as well as field applicability. One such promising test could be development of diagnostics using recombinant Lig proteins of *Leptospira*, since these proteins appear early in the infection and induce strong antibody response. These proteins are expressed only by pathogenic and not by saphrophytic *Leptospira*. The leptospiral immunoglobulin like proteins (Lig) are encoded by genes such as *ligA*, *ligB* and *ligC*. 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.

Expression of recombinant *lig*B proteins

DNA was extracted from *Leptospira borgpetersonii* Hardjo serovar and used for amplifying the regions of gene sequences of *ligB*. The conserved *ligB*con2 (883-1935 bp) and variable *ligB*var (1966-3096) regions of *ligB* gene were amplified by PCR, which resulted in specific amplification. Further, the amplified PCR product of variable region of *ligB* gene (*ligB*var) was sub-cloned into pET33b vector at *NotI* sites and conserved ligBcon2 in pET32a vector at *BamHI* sites respectively. Cloned inserts along with vectors were then introduced into BL21 *E. coli* cells for expression. The expressed recombinant proteins were subjected to SDS-PAGE and characterized. On analysis, bands of ~47 kDa for *ligB*var and ~57 kDa for ligBcon2 has been observed, which is co-relating well with predicted size of 39 & 36 kDa for *ligB*var and ligBcon2 respectively with the fusion protein in the vector.

Extraction of OMP proteins of *Leptospira*

OMP proteins from *Leptospira* were extracted from *Leptospira borgpetersonii* Hardjo to use it as for comparative control. Leptospiral culture containing the organisms at the concentration of 4 x 10^{10} was washed twice in PBS with 5mM magesium chloride. Then the pellet was extracted with 1% protein grade Triton X-114, 150mM NaCl, 20mM Tris (pH8.0) and 2mM EDTA at 4^0 C on a rotating platform for 4hrs. The insoluble material was removed by centrifugation at 17,000 x g for 10 min. Calcium chloride was added to half of the supernatant and the pellet was discarded. Phase partitioning was carried out by incubating at 37^0C for 10 min in an orbital shaker to separate the phases. A lower Triton X-114 phase and an upper aqueous phase were formed. Upper aqueous phase was removed by centrifuging at 1000 rpm for 10 min. Lower Triton X -114 phase was decanted and washed twice with 20% of is volume in10mM Tris (pH7.5)-5mM EDTA to remove any aqueous material remaining at the detergent-aqueous inter phase and centrifuged at 2000 rpm for 10 min at room temperature. The detergent phase proteins

were precipitated with 10 volumes of acetone on ice for 45 min. Then the pellet was air dried and reconstituted in PBS (pH7.4) .Then the OMP pellet was reconstituted in PBS and stored at -20°C. The OMP thus extracted will be further characterized by SDS-PAGE.

Standard serum samples

During this period under report, 398 serum samples (Human-55, Cattle-262, Pig-61, Sheep-9 and Goats-11) have been collected from Karnataka and Kerala and MAT results for these sera were made available, for using these serum samples as known status in respect to *Leptospira* antibody. Further work on expressed recombinant proteins as antigen in immuno-assay like ELISA for detection of *Leptospira* antibodies in bovine samples is in progress.

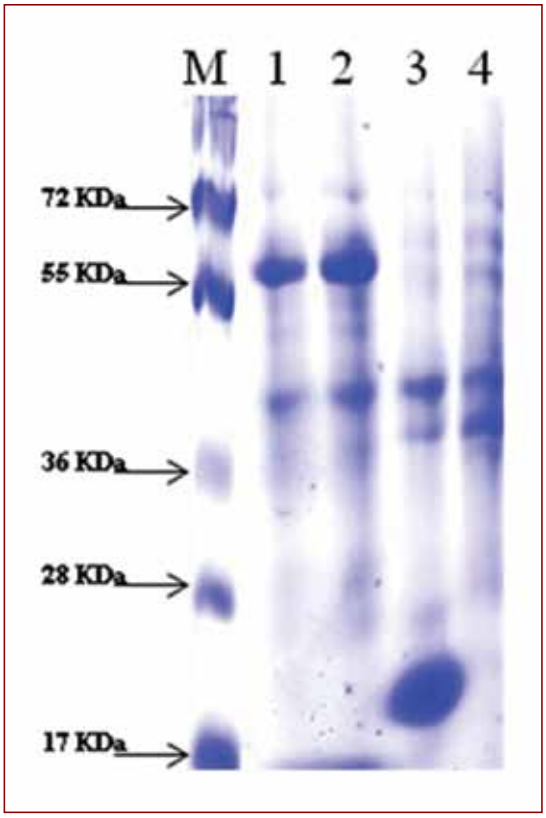


Fig. 25. SDS-PAGE profile of recombinant LigB protein (rLigBcon2); M-Maker Lane 1 & 2 - Bacterial cell lysate from recombinant LigBvar clone 1 and 5; Lane 3-Vector pET32(a) control ; Lane 4-BL21 host cell

Surveillance of methicillin resistant staphylococci and Beta-lactamase producing *Enterobacteriaceae* in animals and their environment

B R Shome and R Shome

Beta-lactamase producing *Enterobacteriaceae*

The growing increase in the rates of antibiotic resistance is a major cause for concern in *Enterobacteriaceae* family, primarily *Escherichia coli*. Currently, the most attention receiving beta-lactamases are the extended-spectrum beta-lactamases (ESBLs), inhibitor-resistant beta-lactamases and carbapenemases. In the present investigation total 294 milk samples were collected from 4 organized farms and 3 unorganized herds. All the milk samples were then subjected to somatic cell count and electrical conductivity analysis. After SCC and EC analysis 1ml of each milk sample was enriched with 5ml EC broth. Subsequently a loop full of enriched milk sample was streaked in Mac conkey agar and kept for incubation at 37°C for 18 hours. The pink color colonies were further purified by subculturing on Mac Conkey agar. These were subjected for Gram's staining. The observation of gram negative rods tentatively identified them as *E. coli*. Further a pure culture was streaked on nutrient agar, after 20-24 hrs of incubation the culture were confirmed by mPCR based approach, targeting five housekeeping gene: ***uid*A** (ß-D-galactosidase), ***lac*Z** (ß-D-galactosidase), ***lac*Y** (lactose permease), ***cyd*** (cytochrome bd complex) and ***pho*A** (bacterial alkaline phosphatase). Total of 99 isolates were confirmed to be *E. coli* by this standardized mPCR. Additionally 71 tentatively identified *E. coli* samples from Sikkim were procured as a part of surveillance of antimicrobial pattern in farm animals (cattle and poultry) prevalent in India. The isolates were streaked on Mac conkey agar to get pure lactose fermenting colonies; mPCR confirmed 55 isolates to be *E. coli*.

Extended spectrum β-lactamases (ESBLs) are enzymes produced by bacteria that can degrade and confer resistance to some of the most commonly used antibiotics including penicillins, cephalosporins and monobactams (Bonnet, 2004). They are a rapidly evolving group of beta lactamase enzyme produced by gram negative bacteria, which have the ability to hydrolyze all cephalosporins and aztreonam but are inhibited by clavulanic acid. These are plasmid - mediated enzymes capable of hydrolyzing and inactivating a wide variety of β-lactams that can be easily transferred between and within bacterial species. Some ESBL genes are mutant derivatives of established plasmid-mediated β-lactamases (e.g., blaTEM/SHV), and others are mobilized from environmental bacteria (e.g., blaCTX-M) (Overdevest et al., 2011).

New Delhi metallo-beta-lactamase 1 (NDM-1) is a newly-described metallo-beta-lactamase (MBL), first identified in 2008 in single isolates of *Klebsiella pneumonia* and *Escherichia coli*. Like other acquired MBLs, NDM-1 hydrolyses all beta-lactam antibiotics except for aztreonam, which is usually inactivated by co-produced extended-spectrum or AmpC beta-lactamases. An association with other resistance mechanisms makes a majority of *Enterobacteriaceae* with blaNDM-1 extensively resistant to antibiotics and susceptible only to colistin and, less consistently, tigecycline. Recent studies describe the occurrence of ESBL-producing *Enterobacteriaceae* in meat, fish, and raw milk; therefore, the impact of food animals as reservoirs for and disseminators of such strains into the food production chain must be assessed.

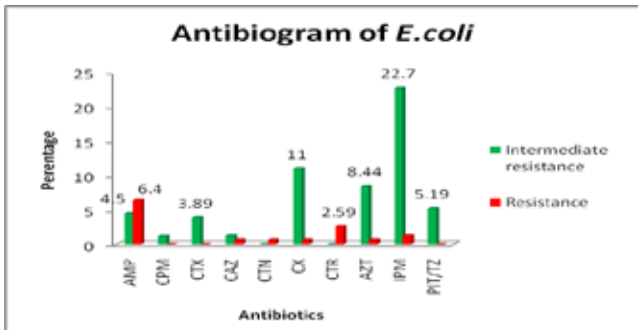
Considering this, a pilot scale study was conducted where 154 (99 Milk and 55 Fecal) *E.coli* samples were screened for antimicrobial susceptibility by Kirby-Bauer disc diffusion method for 3rd and 4th generation cephalosporins, carbapenems and monobactums. The organisms were subjected to antimicrobial susceptibility testing on Muller Hinton agar plates by disc diffusion method using commercially available hexa-disc and individual discs (Hi-media) of Ampicillin (10mcg), Cefotaxime (30mcg), Ceftazidime (30mcg), Ceftriaxone (30mcg), Cefoxitin (30mcg), Cefotetan (30mcg), Aztreonam (30mcg), Imipenem (10mcg) and Piperacillin/ Tazobactum(100/10mcg). The isolates exhibiing intermediate resistance and resistance by disc diffusion method, were employed for confirmation by Modified Hodge test (MHT), Double Disk Synergy Test (DDST) and Inhibitor-Potentiation Disc Diffusion Test (IPDD).

The MHT was performed by preparing a 0.5 McFarland dilution of the *E. coli* ATCC 25922, in broth or saline. A lawn of *E.coli* ATCC 25922 was streaked with the help of a sterile swab and allowed it to dry. Then a disk of meropenem and imipinem was placed at the center and test organisms were streaked in a straight line from edge of the disc to the edge of a plate and kept for incubation at 37^{o}C for 20-24 hrs. DDST was performed to determine the synergy between discs of amoxicillin/clavulanic acid (CA) (20/10mcg) and 30mcg discs of ceftazidime and cefotaxime placed at distance of 15mm. The plates were incubated for 18-20hrs at 37^{o}C. Similarly IPDD was used to confirm the presence of ESBL positive isolates by placing a disc of ceftazidime (30mcg) and cefotaxime (30mcg) alone and ceftazidime and cefotaxime in combination with CA (30/10mcg) atleast 20m apart from each other.

ESBLs and MBLs producing isolates were further confirmed by E-test for Imipinem/ Imipenem EDTA synergy, Meropenem, Triple ESBL detection strip (Ceftazidime, cefotaxime and cefepime mix+clavulanic acid) and ESBL & AMPc detection strip (Ceftazidime, cefotaxime, cefepime & cloxacillin mix). The tests were performed by preparing a 0.5 Mcfarland dilution of the test organisms and by streaking the entire surface of the plate. After that the strips were placed carefully at the desired position on the plate and transferred the plate into the incubator. All the tests were performed according to recommendations

from the Clinical and Laboratory Standards Institute (2011). Quality control strains used were ATCC 25922 and ATCC 35218, ATCC1706, ATCC 27853 as negative controls; ATCC 1705 and ATCC 700603 as positive controls for the test.

The diameter of the zone of inhibition for each antibiotic was measured and interpreted as per the CLSI guidelines. The present work revealed that resistance to antibiotics were Ampicillin (6.4%), Aztreonam (0.64%), Imipenem (1.29%), Ceftazidime (0.64%), Ceftriaxone (2.59%) Cefoxitin (0.64%) and Cefotetan (0.64%), while intermediate resistance to Ampicillin (4.5%), Cefepime (1.2 %), Cefotaxime (3.89%), Cefoxitin (11.0%), Aztreonam (8.44%), Imipenem (22.7%), Ceftazidime (1.29 %) and Piperacillin/Tazobactum (5.19%).



DDST and IPDD confirmed four isolates to be ESBL producers by showing increase in the zone diameter for either of the antimicrobial agent used in combination with CA versus its zone when tested alone (Table 1).

E-test for ESBL mix and AmpC
Inhibitor-potentiation disc diffusion

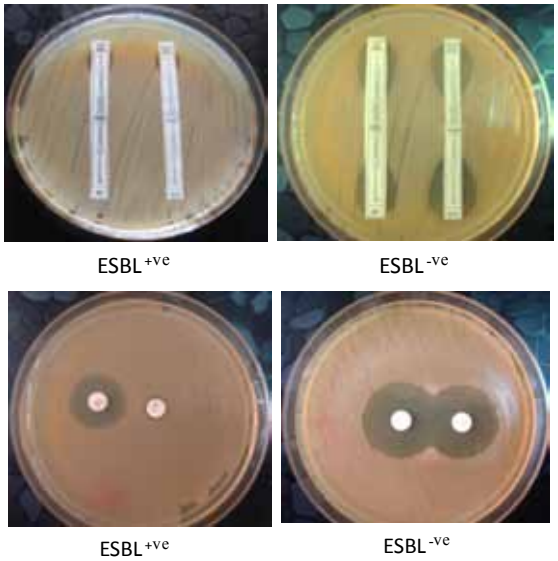


Table 1. Antimicrobial susceptibility test showing profile of the ESBL producing E. coli isolates

Sl. no	Screening by Disc diffusion method								Confirmation for MBLs (carbapenems)			Confirmation for ESBLs (cephalosporins)		
	Cephalosporins					Carbapenems		Ampicillin	MHT	E-test Meropenem	Imipenem (w/wo) EDTA	DDST	IPDD	E-test ESBL and AmpC
	CPM	CTX	CX	CAZ	CTR	MER	IPM							
BF75a	R					IR		R	-	-	-	+	+	ESBL & AmpC +
BF75b	R					S		R	-	-	-	+	+	ESBL & AmpC +
B27	R					S		R	-	-	-	+	+	ESBL & AmpC +
B50	R					S		R	-	-	-	+	+	ESBL + & AmpC -

Carbapenem-hydrolysing β-lactamases such as KPC type, IMP and VIM types and OXAare now reported worldwide among *Enterobacteriaceae*. Recently, a family of ESBLs which preferentially hydrolyze cefotaxime (CTX), the CTX-M-β-lactamases, have been recognized and reported in the literature with increasing frequency (Pitout *et al*, 2004). The class B carbapenemase NDM-1 (New Delhi metallo-b-lactamase) has been identified mostly from *Enterobacteriaceae*, mainly from India, (Bell *et al*, 2007).

So the selected isolates with positive screen tests will be subjected to molecular screening for β lactamases using PCR tests for TEM, SHV, CTX-M, IMP, VIM, NDM1&2, AMP-C using published primers which have been procured (Perez et al, 2002; Pitout et al, 2004; Mendes, 2007 and Kojima, 2005).

In conclusion, together these tools, phenotypic and genotypic study can more accurately detect the increasingly complex resistance mechanism in bacteria present in food producing animals and in humans.

Methicillin resistant staphylococci

A study was undertaken to trace the possible transmission of methicillin resistance between human and animals based on the knowledge gained about the existence of methicillin resistant strains of Coagulase negative staphylococci (CoNS). ABST analysis of 254 staphylococci (40 *S. aureus* and 214 CoNS) revealed that resistance to methicillin was found in 4.21% of CoNS isolates whereas all *S. aureus* isolates were sensitive. Also Minimum Inhibitory Concentration (MIC) was determined for Methicillin for all the 27 *mec*A positive isolates using Hi-Comb (Hi-media, India) method (CLSI 2010). It was found that 77.78% of the isolates were showing resistance to Methicillin by having an MIC value equal to or more than 16µg/ml. The remaining 22.22% were susceptible to Methicillin MIC value less or equal to 8µg/ml.

Screening for the presence of *mec*A gene revealed that 27 CoNS isolate possessed the *mec*A gene (Fig.1). On subtyping of the SCC-*mec* element (Fig.2a & 2b), it was found that the animal *S. haemolyticus* isolates 85.71% (6/7 isolate) and 50% (5/10 isolate) of human (animal handler) *S. epidermidis* isolates carried **type V SCC *mec* element**. In addition, 10% (1/10 isolate) of *S. epidermidis* from animal handler carried a **type VI SCC *mec* element.** The study showed that there was no similar SCC*mec* elements found among the CoNS isolates from animal and human. But, it was found that the milk *S. haemolyticus* isolates and human *S. epidermidis* isolates carried a type V SCC*mec* element, suggesting a possibility of interspecies transmission of SCC*mec* elements.

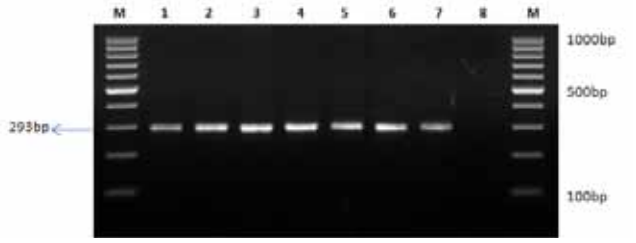


Fig.1. PCR products for *mec*A gene: Lane 1: Positive control of *mec*A gene (*S. aureus* ATCC 43300), Lane 2-7: Methicillin Resistant coagulase negative staphylococcal isolates; Lane 8: Methicillin sensitive coagulase negative staphylococcal isolate; Lane M: Molecular size marker (100bp DNA ladder; Fermentas).

So far eleven types (I to XI) of SCC*mec* have been assigned for *Staphylococcus aureus* based on the classes of the *mec* gene complex and the *ccr* gene types (www.sccmec.org/Pages/SCC_TypesEN.html). There are no definite classification schemes for coagulase negative staphylococci. We have identified some new SCC*mec* elements in isolates of *S. haemolyticus*, *S. epidermidis, S. hominis* and *S. sciuri* which could not be classified based on current schemes and necessitate further study.

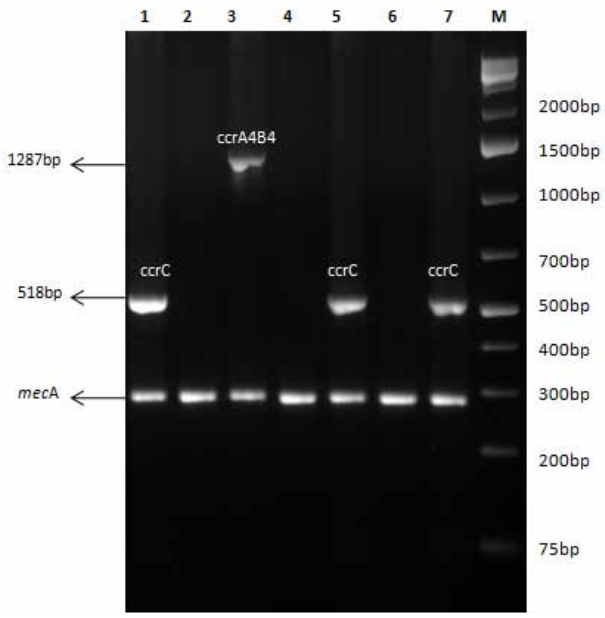


Fig. 2a. mPCR for identification of *ccr* genes for the assignment of the type of *ccr* gene complex: Lane 1,5

and 7: *ccr* type C; Lane3: *ccr* type A4B4, Lane 2,4 and 6: *ccr* genes could not be amplified; Lane M: Molecular size marker (1kb plus DNA ladder; Fermentas); The DNA fragment corresponding to *mec*A served as a internal control in each lane.

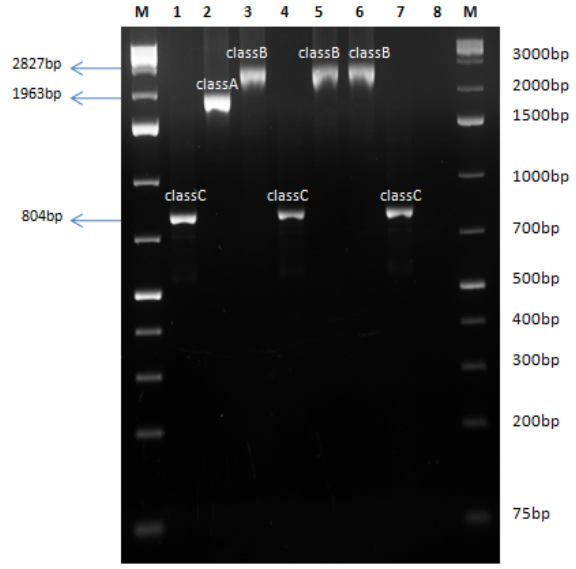


Fig. 2b. mPCR for identification of gene alleles for assignment of the *mec* gene complex: Lane 1,4 and 7: class C *mec*; Lane 2: class A *mec*; Lane 3, 5 and 6: class B *mec*; Lane 8: control negative with nuclease free water; Lane M: Molecular size marker (1kb plus DNA ladder; Fermentas).

It was reported that *S. aureus* ST130 from bovine mastitis in UK and also MRSA ST130 from humans contain a *mec*A homologue mec_{LGA251} which is not detected by PCR established for detection of *mec*A (Cuny et al., 2011). Similarly MRSA isolates from humans in England and in Denmark which were exhibiting MRSA phenotype but negative for *mec*A gene by PCR were found to carry this *mec*A homologue mec_{LGA251}. These *mec*A homologues were found to have 70% homology with the *mec*A gene. In the present study while evaluating the antimicrobial susceptibility of *Staphylococcus* phenotypically, interestingly we could find 11 isolates showing intermediate resistance and resistance profile to Methicillin, but these isolates were not carrying a *mec*A gene. Hence these isolates were taken up for *mec*A homologue mec_{LGA251} screening using the primer pairs previously described by Cuny et al., 2011. But none of these isolates carried a *mec*A homologue indicating that these isolates might possess new *mec*A homologues or other mechanisms leading to β-lactam resistance, hence, full genome sequence analysis is crucial to delineate the responsible genetic elements.

Epidemiological study on trypanosomiasis and fascioliasis in animals

P. P. Sengupta, V. Balamurugan and P. Krishnamoorthy

A total of 247 cattle sera samples were collected from Odisha and were screened for the presence of antibodies against *Trypanosoma evansi* by indirect ELISA. An overall 13.25% samples were recorded as positive. Out of these samples, 0.6 % shown positive from Jajpur, 5.4% from Kendrapara, 2.4% from Puri and 4.75% from Cuttack (Fig. 26).

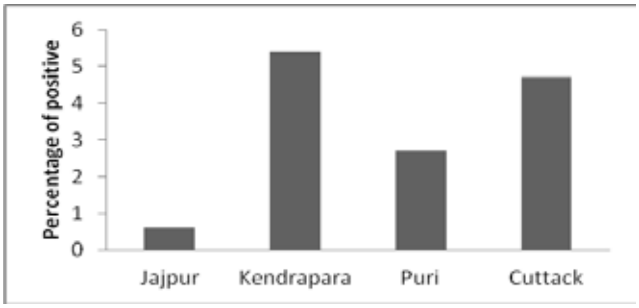


Fig. 2. ELISA screening of serum samples from Odisha for trypanosomiasis.

A total 50 faecal samples collected from cattle in Odisha were subjected for microscopical examination. Out of which, 46.66% were positive for *Oesophagostomum* sp., 36.33% for strongyles and 20% for *Fasciola* sp. infection (Fig. 2). The snails collected from these areas were positive by PCR assay for *Fasciola* sp as high as 75% in Jajpur, 50% in Kendrapara and 100% in Puri were positive for PCR (Fig. 3).

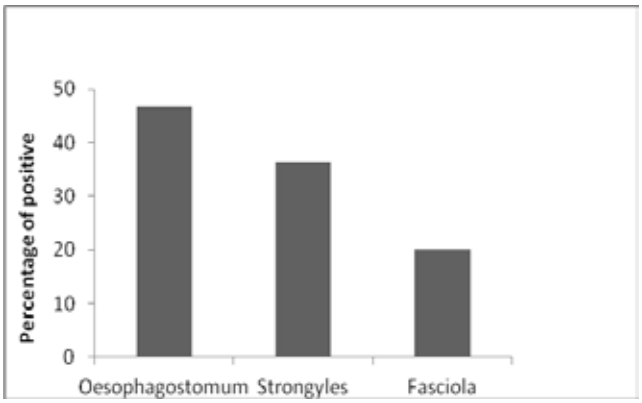


Fig. 2. Microscopic examination of faecal sample of Odisha.

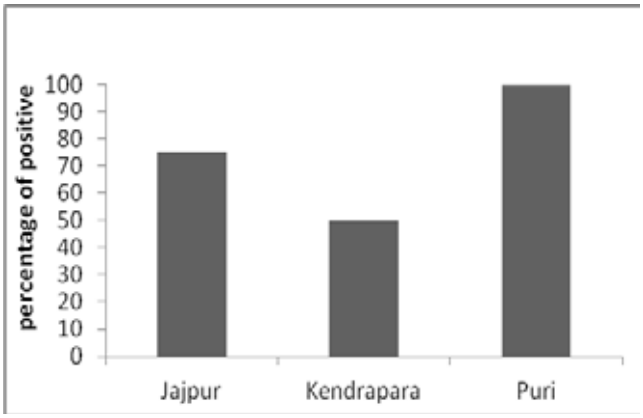


Fig. 3. PCR analysis of snail tissues for *Fasciola* spp.

Chikballapur

A total of 66 sera samples collected from Chikbalapur district, Karnataka and were screened for the presence of antibodies against trypanosomiasis by ELISA. Out of 66 samples only one sample was found as positive.

Kerala

Out of 33 samples collected from Palakkad District of Kerala, one sample was found as positive for the presence of antibodies against *T. evansi* by ELISA.

Sequencing & cloning of ISG gene of *Trypanosoma evansi* and its use in diagnosis

Invariant surface glycoprotein (ISG) gene of *Trypanosoma evansi* isolated from buffalo were cloned and sequenced. Its genome sequence was submitted to GenBank and the accession number is JN797772. This is the first time ISG gene of *T. evansi* reported from Asia. Phylogenetic analysis reveals that the organism is closely related to *T. evansi* RoTat 1.2 and *Trypanosoma brucei gambiense* (Fig.4). A primer pair was designed, aiming ISG genes, which could amplify 407bp DNA product by PCR reaction. The sensitivity of the diagnostic PCR was conducted on experimental animals such as buffalo and rat and detected the parasite in the blood on 24hr pi and 3rd dpi respectively. While the PCR detected 0.04 pg and 1.2 ng of DNA from purified trypanosomes and infected rat blood respectively. The specificity assay with other haemoprotozoon DNA was also conducted, the primer pair amplified the 407 bp DNA from blood of rats infected with other isolates of *T.evansi* such as dog, lion and leopard. The primer appeared highly sensitive and specific in diagnosis of carrier status of *Trypanosoma evansi* infection (Fig. 5 & 6).

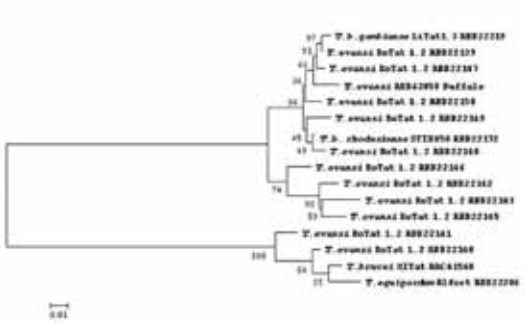


Fig. 4. Bootstrap consensus of ISG from different isolates / species based on deduced amino acid sequences of ISG protein using neighbor joining tree method of the MEGA 4 version. The branch values represent the bootstrap confidence values using 1,000 replicates of the data set. The bars represent the genetic distance.

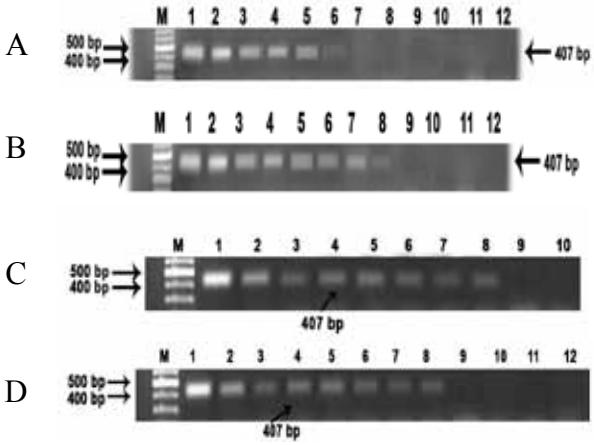


Fig. 5. Sensitivity assay by dilution: A and B: Infected rat blood (trypanosomes (ml-1)) in PBS and healthy buffalo blood C and D: Purified trypanosomes (ml-1) in healthy buffalo blood and PBS

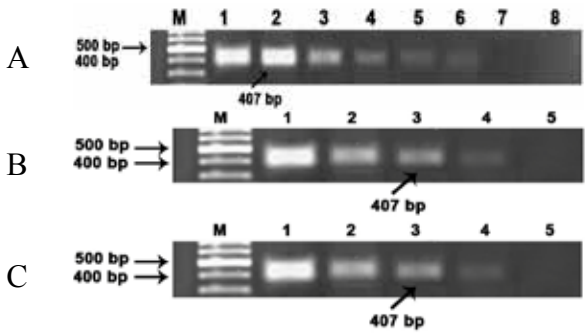


Fig. 6. Specificity assay with infected rat tissuesA: T.evansi (dog isolate) B: T.evansi (lion isolate) C: T .evansi (leopard isolate)

Analysis of cumulative disease data

The disease data available from NADRES during the period of 1990 – 2011, were analyzed to know the disease status.

Among cattle, buffaloes, sheep and goats, ascariasis appeared as the highest occurring parasitic disease followed by strongylosis, fascioliasis, babesiosis, amphistomiasis, trypanosomiasis, theileriosis and so on (Fig. 8). Trypanosomiasis caused highest death followed by amphistomiasis, fascioliasis, theileriosis, babesiosis and so on (Fig. 9).

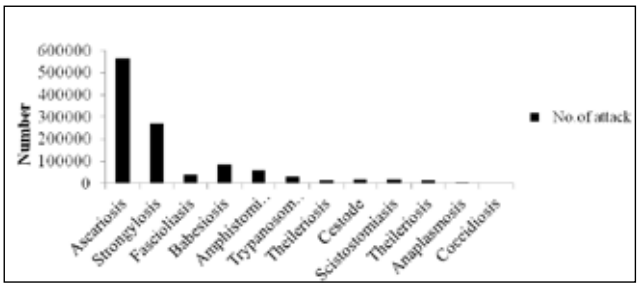


Fig. 8. Ranking parasitic diseases based on no of attacks during 1990-2011.

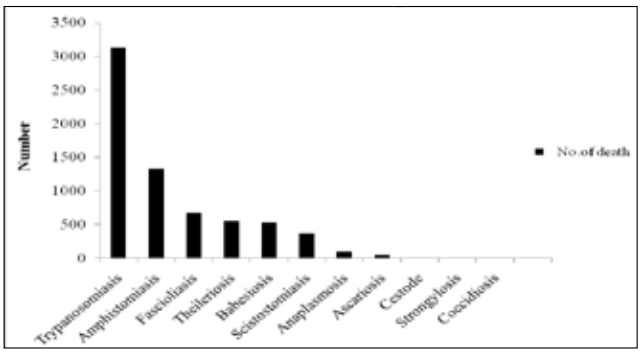


Fig. 9. Ranking of parasitic diseases based on mortality in livestock during 1990-2011.

In cattle, ascariasis was the highest prevailing disease followed by strongylosis, babesiosis, amphistomiasis, trypanosomiasis, theileriosis and so on (Fig. 10). Whereas, trypanosomiasis caused maximum mortality followed by amphistomiasis, babesiosis, theileriosis, fascioliasis and other diseases (Fig. 11).

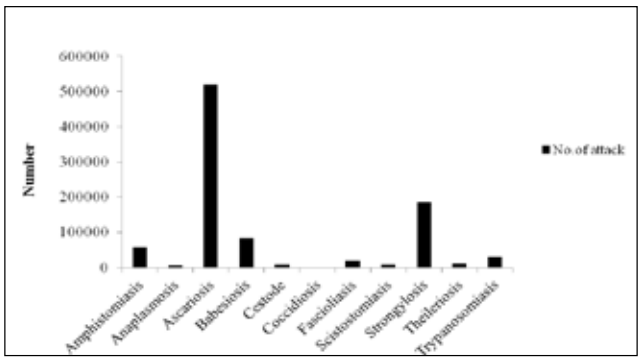


Fig. 10. Ranking of parasitic diseases based on prevalence in cattle during 1990-2011.

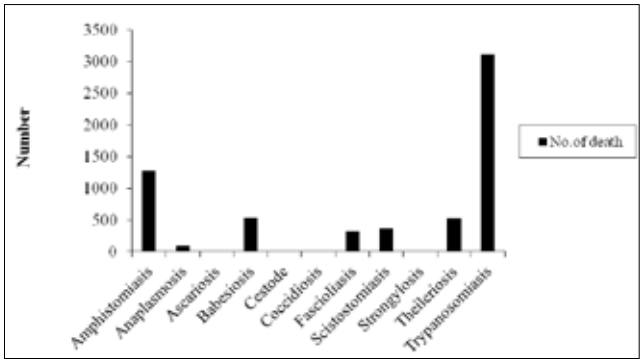


Fig. 11. Ranking of parasitic diseases based on mortality in cattle 1990-2011.

Among buffaloes, ascariasis was found as the highest occurring disease followed by strongyliasis, fascioliasis, babesiosis, trypanosomiasis and other diseases (Fig. 12), however, the highest death caused due to fascioliasis, ascariasis, paramphistomiasis, trypanosomiasis, theileriosis and so on (Fig. 13).

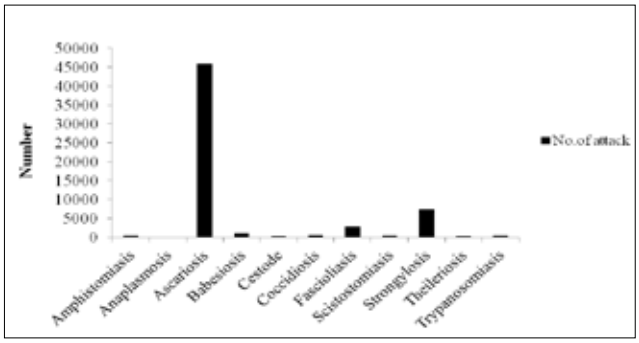


Fig. 12. Ranking of parasitic diseases based on prevalence in buffalo during 1990-2011.

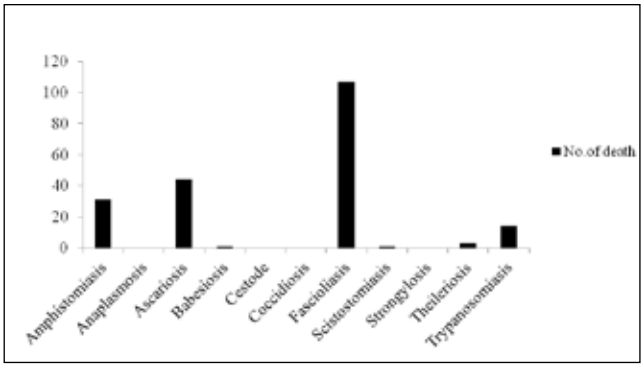


Fig. 13. Ranking parasitic diseases based on mortality in buffalo during 1990-2011.

In goats, strongyliasis was the highest occurring disease followed by fascioliasis, coccidiosis,cestode infection, babesiosis and so on (Fig.14). Whereas fascioliasis caused highest mortality followed by amphistomiasis, theileriosis, coccidiosis and other diseases (Fig. 15).

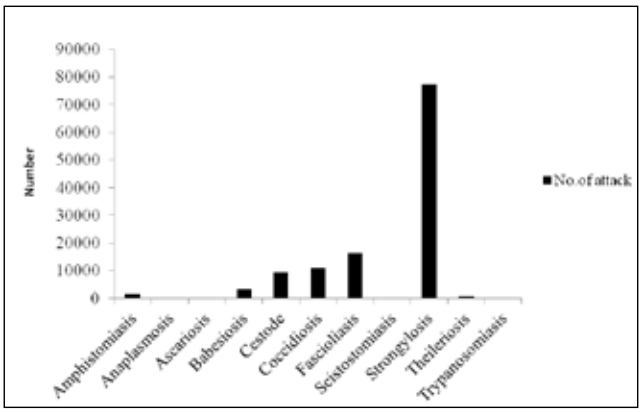


Fig. 14. Ranking of parasitic diseases in goat based on prevalence during 1990-2011.

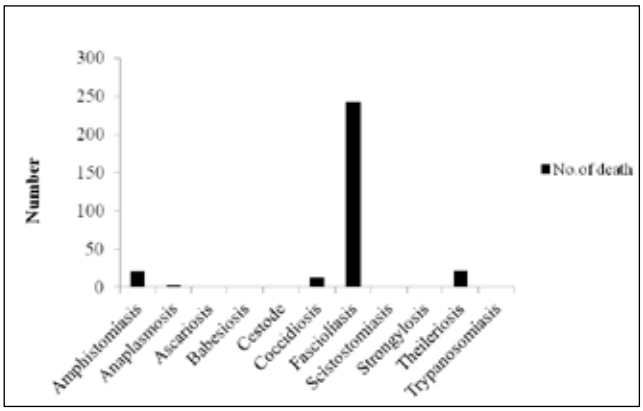


Fig. 15. Ranking of parasitic diseases based on mortality in goat during 1990-2011.

Death occurred due to trypanosomiasis in cattle was found highest in October i.e. post monsoon season when vector population in the environment was maximum after breeding and lowest was in December in dry winter season (Fig. 16).

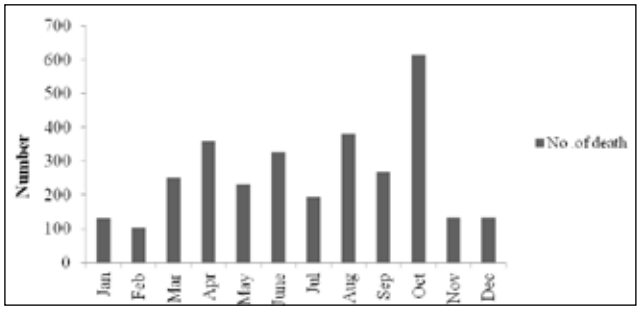


Fig. 16. Monthwise mortality due to trypanosomiasis in cattle during 1990-2011.

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

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

Sample survey of organized dairy farms was carried out and selected organized cattle farms in Hubli, Dharwad, Bijapur in Karnataka and Pondicherry, Chennai in Tamilnadu for the study. The farms were having intensive system of rearing with stall feeding except Dharwad which is having semi range system of rearing. Paired serum samples with gap of one month apart 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, it was found 254 (44%) and 158 (27%) positive respectively. The serum minerals like copper, zinc, calcium, phosphorus and magnesium were estimated. The serum copper, zinc, calcium, magnesium and phosphorus were 1.35 ppm, 1.52 ppm, 10.88 mg%, 4.65 mg% and 2.67 mg% respectively in apparently healthy animals. 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 concentrate was 14.98, 3.63 and 12.45 MJ/kg and fodder was 9.98, 1.06 and 4.73 respectively. The values obtained for the cattle feed were within the normal range. Soil samples were analyzed for trace minerals like copper, zinc and molybdenum. The soil analysis for mineral levels varied widely depending on the type of the soil in the organized farms. Economic analysis of the data showed the reproductive disorders are the major factor causing loss to the farms. In which, the repeat breeding and abortion were causing the significant economic losses in the organized dairy farms.

External Funded Projects

All India Network on Bluetongue

Divakar Hemadri

As suggested during the previous annual workshop on bluetongue, the PD_ADMAS center started working on countrywide epidemiology (both spatial and molecular) of bluetongue rather than within the earlier jurisdiction of Karnataka state. Because of the fact that PDADMAS is a non funded center, and has no technical support to carry out the objectives of the project, it was decided to take up the work in a phasewise manner. As PDADMAS center was already having bluetongue disease information for the past 15 years, it was decided to start spatial epidemiology work with the state of Karnataka. Given below are the details of research work carried out at PDADMAS during 2011-12.

Spatial Epidemiolgy

Updating of digital maps

As per the 2011 census, the number of districts in the country has gone up from 597 to 640. As a consequence the district wise GIS map of the country is required to be updated. Updating means manual digitization of newer districts within each state. Since the work involved is huge, it was decided to take up this work also in the phasewise manner. As a first step, updated GIS maps of Karnataka and Tamilnadu state were prepared. The work involved digitization of six districts (number of districts increased from 27 to 30) in Karnataka and five districts in Tamil nadu (up from 30 to 32). The newly updated GIS maps of Karnataka and Tamil nadu are given below (Fig. 1), which shows updated districts within the circle.

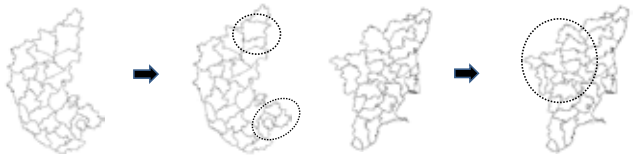


Fig. 1. Digital maps of Karnataka and Tamil nadu. Newly created districts are shown within the circle

Creation of a database on bluetongue disease outbreaks

For the creation of bluetongue disease database, information obtained from Department of Animal Husbandry, Government of Karnataka was used. The department compiles disease information such as number of animals at risk, no affected and no. died at the district level. In other words, the maximum resolution of the data available is at district level. Luckily, for more than 50% of the outbreaks, information on the villages/blocks affected were available and the same was used for spatial analysis. The bluetongue disease database for Karnataka presently contains information about 2500 outbreaks spanning the past 15 years.

Creation of countrywide livestock population database

Spatial epidemiology involves risk analysis. Therefore for carrying out risk analysis, a database containing various disease precipitating factors such as land use and land cover, soil type, siol moisture, surface temperature, wind speed and wind direction, susceptible livestock population etc. suitable for spatial analysis is required. As a first step towards this work, block level information on livestock population for the entire country was downloaded from the website of DADF, GOI and a database suitable for spatial analysis for the state of Karnataka was created. Given below (Fig. 2) is the density map of sheep and goats in Karnataka. Lowest density is shown in yellow colour whereas the highest density is shown in deep purple colour.

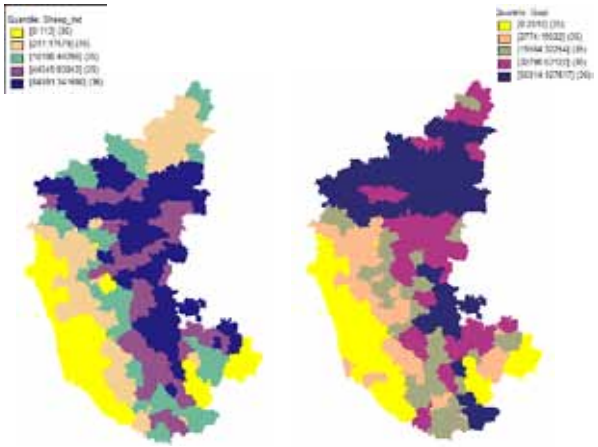


Fig. 2. Density map of sheep and goats in Karnataka. Yellow colour indicates lowest density while deep purple indicates highest density.

Extraction of geographical coordinates

Disease mapping involves true geographical representation of place of outbreaks on the maps. A true disease map can then be used for spatial analysis and cluster detection. As names of villages affected were available for nearly 50% of the outbreaks, an attempt

was made to represent the same on the map. For the purpose geographical representation, geographical coordinates for affected villages were extracted from various internet sources and plotted on the map. Given below (Fig. 3) is the preliminary bluetongue disease map for the state of Karnataka for the past 15 years. The map shows higher incidence of the disease in the southern region of the state, although the northern region has higher sheep population. The reasons could be vicinity of veterinary institution, progressive nature of the farmers in southern region, nomadic nature of the sheep farmers in the northern region.

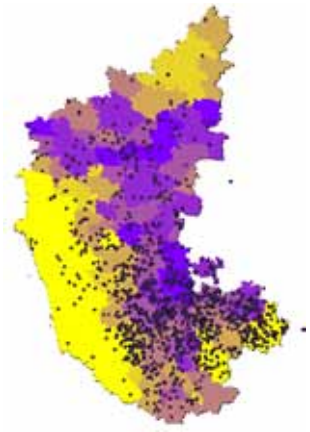


Fig. 3. Bluetongue disease map of Karnataka state.

Analysis of disease maps

Bluetongue disease map for the period 2007-2011 is given below (Fig. 4). The map shows occurrence of the disease in 9 districts of the state, with highest number of outbreaks being reported from Tumkur district, followed by Chitradurga and Bagalkot districts.

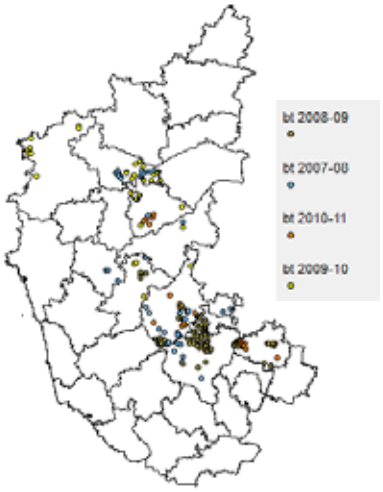


Fig. 4. Bluetongue disease map for the period 2007-2011

Year 2007-08 (Fig. 5)

The disease first appeared in Pavgada taluk (encircled by AP) in september continued till october. Later on the disease spread to neighbouring taluks in Chitradurga and Tumkur districts. The disease outbreaks continued engulfing the newer areas. It is not clear from the existing data that whether the disease has spread from south to north or north to south as outbreaks were recorded Hungund taluk in November. Serotype information on these outbreaks may further help to resolve the issue further.

2007-08 **2008-09**

Fig. 5 **Fig. 6**

Year 2008-09 (Fig. 6)

Comapared to 2007-08, the disease was less widespread this year. The disease first appeared in various places in Tumkur and Chikballapur districts in October and curiously after causing series outbreaks in Sira taluk of Tumkur, Chitradurga, Davanagere and Chikballapur districts, the disease died out. The reason for this behaviour needs further investigation.

Year 2009-10 (Fig. 7)

In the year 2009-10, the disease was mostly reported from northern districts of the state, apparently due to higher precipitation and floods. The disease which was started in October was in full bloom during November and December. Interestingly the southern districts did not report the disease indicating the cyclical nature of the disease.

2009-10 **2010-11**

Fig. 7. **Fig. 8.**

Year 2010-11 (Fig. 8)

The disease returned to the southern districts, with comparatively lesser number of outbreaks being reported. Interestingly the disease continued for longer period of time.

Although most of the analysis done in the present study is preliminary, never the less the work in this direction has given boost to spatial epidemiology of the disease in India.

Molecualr Epidemiology

During the year under report, some of the clinical samples collected from Chitradurga and Chikballapur districts between 2010 and 2011 were subjected to PCR. All the eight samples analysed were tentatively typed as serotype 2. Then the PCR products were subjected to restriction endonuclease analysis, which further confirmed that all the products indeed belong to serotype 2. To support the above findings, the PCR products were nucleotide sequenced. So obtained nucleotide sequences were aligned with that of 23 other serotypes and used for construction of phylogenetic tree (Fig. 9) using neighbour joining method. From the genetic analysis, it was clear that the viruses from Chitradurga and Chikballapur are about 96-97% genetically similar to Mehboob nagar and Tirupati isolates and are different from South African reference and European isolates. Study also showed that source of infection for both the above outbreaks were different, although, both the isolates belonged to eastern topotype.

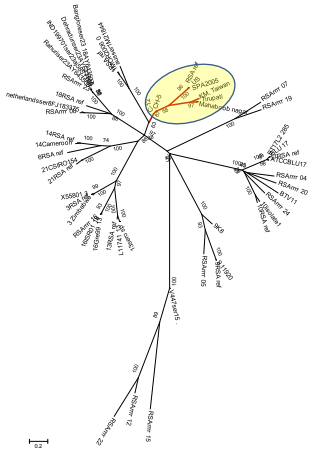


Fig. 9. Neighbour joining tree showing the grouping of Chitradurga (CH-5) and Chikballapur (C-12) with other BTV-2 isolates.

Outreach Programme on Zoonotic Diseases (OPZD)

Rajeswari Shome, V. Balamurugan and M. Nagalingam

Brucellosis investigation

In the OPZD project, sera from risk group and livestock species from various farms were collected and tested for brucellosis. A total of 219 sera samples were collected from farm workers and veterinary doctors and screened for anti-*Brucella* antibodies. Nine out of 219 (4.1%) and 31 out of 219 (14%) were positive by RBPT and ELISA, respectively. All the serum samples were PCR negative. Similarly a total of 753 sera were collected various livestock species and 51/753(6.7%) and 95/753 (12.6%) were positive by RBPT & ELISA, respectively (Table 1). The study clearly indicated the prevalence of brucellosis in both livestock and humans.

Table 1. Species wise screening results for brucellosis

Species	Number of samples	RBPT Positive	ELISA Positive
Cattle	403	32 (8%)	50 (12.4%)
Horse	75	9 (12%)	11 (14.6%)
Sheep	9	0	0
Goat	47	1 (2.1%)	3 (6.3%)
Total	**753**	**51 (6.7%)**	**95 (12.6%)**

Sera samples received from NIMHANS, Bangalore were tested during the period and 6 out of 89 (6.7%) samples were positive by both RBPT & ELISA. Whereas the active infection was confirmed by PCR in 3 out of above 6 (3.3%) sero-positive patients (Table 2). The study clearly indicated the prevalence of active neuro-brucellosis infection responsible for neuropathy and related symptoms in suspected patients.

Table 2. Screening results of brucellosis in human samples received from NIMHANS, Bangalore.

Type of the sample	No. of samples	RBPT	ELISA	PCR
Serum	30	6	5	1
Plasma	29	ND	ND	0
CSF	30	0	1	2
Total	**89**	**6 (6.7%)**	**6 (6.7%)**	**3 (3.3%)**

ND: Not done

Listeriosis investigation

Clinical samples from animals and human were analyzed for *Listeria by* isolation, PCR and ELISA. Samples were collected from both man and animal sources and subjected to diagnosis of listeriosis. Out of 96 samples processed, only 2 isolates could be recovered, which could be confirmed by bacterial identification kit and genus and species specific PCR. ELISA results on the cattle and goat sera samples obtained from listeriosis suspected farms is given below. (Table 3).

Table 3. Details of samples tested for listeriosis.

Source	No. of samples	Isolations	ELISA positive
Human	13	0	0
Cattle	62	1	5
Goat	11	1	2
Sheep	9	0	0
Silage	1	0	0
Total	**96**	**2**	**7**

Leptospirosis investigation

During the period, samples from animals and humans were collected and analyzed for prevalence of Leptospira by isolation and PCR. Out of 198 samples processed, 37(18.6%) isolates could be recovered from rats, dogs, tiger and goat samples. Similarly, 17 out of 198 samples (8.5%) were Leptospira-PCR positive and these samples included human, cattle and dog (Table 4).

Table 4. The details of isolation and PCR results for leptospirosis.

Species	No. of samples	No. of isolates	No of samples	PCR positives
Cattle	75	-	64	8
Rat	18	16	-	-
Goat	13	5	13	0
Dog	62	11	6	2
Sheep	-	-	14	0
Human	20	0	32	7
Tiger	10	5	-	-
Total	**198**	**37 18.6%)**	**129**	**17 (8.5%)**

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

B. R. Shome, P. Krishnamoorthy, R. Shome

A study was undertaken to trace the possible transmission of methicillin resistant Staphylococci between human and animals based on the knowledge gained about the existence of methicillin resistant strains of Coagulase Negative Staphylococci (CoNS). A total of 117 samples were collected from the cows that harbored methicillin resistant strains of CoNS previously and from the animal handlers' and their family members. The samples collected from the animal were milk, swabs of the extra-mammary sites (teat skin, udder surface, perineum and wound), from environment of the animal (Milking machine-teat cup liner and feed trough) and those from the human included swabs of animal handler's hand and external nares and also swabs of the external nares of their family members. The samples were subjected for the

isolation and identification of *Staphylococcus* spp. From 117 samples, a total of 174 staphylococci 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 *Staphylococcus* spp. were *S. sciuri* (n=61), *S. epidermidis* (n=47) followed by *S. aureus* (n=40), the detailed distribution of the different species among the human and animal samples are detailed in Table 1.

Table 1. Details of the *Staphylococcus* spp. isolated from different sources of samples.

Organisms	Human isolates			Animal isolates					Environment		Total
				Milk	Extra-mammary site						
	Animal Handlers		Others		Udder skin	Teat apices	Perineum	wound	Teat cup liner	Feed trough	
	Hand	Nares									
S. aureus	3	1	11	5	6	3	6	-	4	1	40
S. chromogenes	4	-	1	-	2	3	3	-	-	-	13
S. epidermidis	8	10	12	8	3	3	-	-	1	2	47
S. sciuri	17	-	2	10	5	8	4	2	3	10	61
S. haemolyticus	-	-	3	6	1	-	-	-	2	-	12
S. hominis	-	-	1	-	-	-	-	-	-	-	1
Total											**174**

Antibiotic resistance analysis of *Staphylococcus* spp. by disc diffusion method

All CoNS and *S. aureus* isolates were sensitive to Oxacillin, CoNS showed sensitivity towards cephalothin too. Further, the isolates showed varied resistance to other antibiotics, the maximum resistance was towards antibiotics viz. Penicillin (49.07% CoNS; 42.5% *S. aureus*), Ampicillin (43.93% CoNS, 32.5% *S. aureus*), Amoxicillin (18.22% CoNS; 22.5% *S. aureus*) and Enrofloxacin (3.74% CoNS).

Standardization of biofilm production potential in *S. epidermidis* isolates

A multiplex PCR has been standardized for the detection of biofilm producing *S. epidermidis* isolates by targeting genes such as *ica*A, *ica*B, *ica*C, *ica*D (Fig.1). Further, mPCR standardization of genes, which control the transcriptional regulation of *ica* locus (*ica*R and *sar*Z) and major biofilm accumulation protein (*bhp*) of *S. epidermidis* has been achieved (Fig. 2).

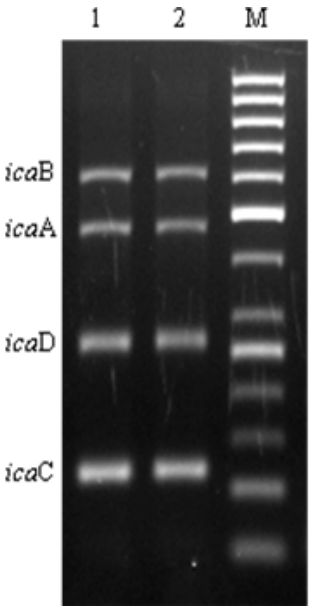


Fig.1. mPCR standardized for the gene *ica*A(475bp), *ica*B(620bp), *ica*C(117bp) and *ica*D(265bp) of the *ica* locus. Lane 1: ATCC 35983 *S. epidermidis*, Lane 2: ATCC 35984 *S. epidermidis*, Lane M: 50bp DNA ladder.

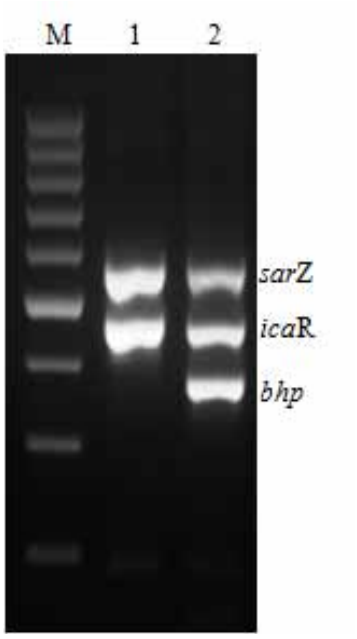


Fig. 2. mPCR standardized for the gene *sar*Z (575bp), *ica*R (496bp) and *bhp* (382bp). Lane M: 50bp DNA ladder, Lane 1: ATCC 35983 *S. epidermidis*, Lane 2: ATCC 35984 *S. epidermidis*.

Investigation of accessory gene regulator (*agr*) genes of *S. aureus* isolates

There was predominance of *agr* type-1 (84.8%, 151 /178) among the *S. aureus* isolates from bovine milk while *agr* type-I and *agr*-III were equally found among human (40%, 6/15) isolates. Although the number of samples collected from extra-mammary sites was scanty, it was found that *agr* type-III (73.3%, 11/15) was predominant among these *S. aureus* isolates (table 2). Interestingly, majority of the bovine isolates of the present study possessed *agr*-type I suggesting the prevalence of strains of persistent nature in the region.

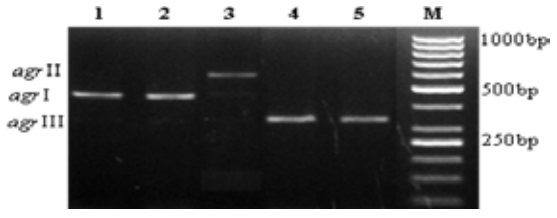


Fig.3. mPCR standardized for the detection of *agr* gene of *S. aureus*. Lane 1-2: *S. aureus* field isolate of *agr* type-I (441 bp), Lane 3: *S. aureus* field isolate of *agr* type-II (575 bp), Lane 4-5: *S. aureus* field isolate of *agr* type-III (323 bp), Lane M: 50bp DNA ladder

Table 2. Distribution of *agr* types among the *S. aureus* isolates from different sources

Isolates origin (No. of isolates)	Accessory global gene regulators			
	***agr*I**	***agr*II**	***agr*III**	***agr*IV**
Milk Isolates (173+5)	151	9+3	9+2	-
Extra-mammary sites (15)	1	1	11	-
Environmental (5)	-	2	1	-
Human (15)	6	-	6	1

Genetic diversity analysis of major mastitis pathogens *S. aureus* by staphylococcal protein A (*spa*) typing

Analysis (40 *S aureus* isolates) revealed that *spa* types t7286 and t002 were predominant in the bovine milk samples, t948 was the predominant *spa* type in the isolates from extra-mammary site (81.82%, 9/11). The *spa* type t948 was also found in isolates from animal environment (teat cup liner) in addition to type t7286. *spa* type t479 was found in 33.33% of human *S. aureus* isolates. The other *spa* types of human *S. aureus* were t2986, t2313 and t3197. No identical *spa* types were found among *S. aureus* isolates from human and bovine milk samples. Also, three new *spa* types have been identified which are to be submitted to the Ridom *spa* server.

MLST analysis of *S. aureus*

S. aureus isolates of *spa* type t267 (2 isolates), t359 (2 isolates) and t6877 (1 isolate) were subjected for MLST analysis. The isolates with *spa* type t267 and t6877 belonged to unique new sequence types viz. ST-2182 and ST-2219 respectively. Of the two isolates of *spa* type t359 analyzed, one isolate was of ST-2182 and other isolate possessed a new *pta* allele and hence a new ST 2368 was designated by the curator.

MLST analysis of *S. epidermidis* isolates

Majority of the bovine milk origin *S. epidermidis* isolates (58.33%, 7/12) were of type ST-114. The other sequence types of milk isolates were ST-329 and ST-54. Human isolates were distributed

among ST-329, ST-210, ST-179, ST-59 and ST-57 (Table 3). ST-329 was found among the animal handler and bovine milk isolate of *S. epidermidis*, and interestingly, the isolates were from different herds. Further epidemiological significance of ST-329 could not be established. Also, ten new alleles and three new allelic combinations were found among the *S. epidermidis* isolates. These new profiles have been submitted to the curator of *S. epidermidis* MLST database.

Table 3. Distribution of sequence types among the *S. epidermidis* isolates obtained from different sources

Source of *S. epidermidis*	ST-329	ST-210	ST-179	ST-114	ST-59	ST-57	ST-54	New
Bovine milk (12)	1	-	-	7	-	-	1	3
Human (13)	2	1	1	-	1	2	-	6

Pulsed-field gel electrophoresis (PFGE) analysis of bacterial isolates

***Streptococcus* spp. –** A total of 17 *S. agalactiae* and 13 *S. uberis* isolates were subjected to PFGE analysis to determine the epidemiological relationships among the strains.Variability in the band pattern of 17 *S. agalactiae* isolates (A, A_1 and A_2) from the same herd was observed (Fig.4), though a single PFGE type (A) predominated. The distribution of band types were: A in 13 (76.47%), A_1 in 3 (17.65%) and A_2 in only one (5.88%) of the *S. agalactiae* isolates. The 13 *S. uberis* isolates were distributed among four unique patterns (A, A_1, B and C) (Fig.5). Two band types A and A_1 were found in three and two isolates of ST-439, with up to six band difference; band type B was found in all the *S. uberis* isolates of ST-475 and band type C was found in the only *S. uberis* isolate of ST-474. Similar or identical patterns were found within the same herd and in no instance were identical PFGE patterns observed for cows from different herds. Previous studies have also reported identical restriction pattern for *S. agalactiae* isolates within the same herd (Khan et al., 2003; Merl et al., 2003). It was also reported that chronically infected cows often harbour the same PFGE type (Phuektes et al. 2001).

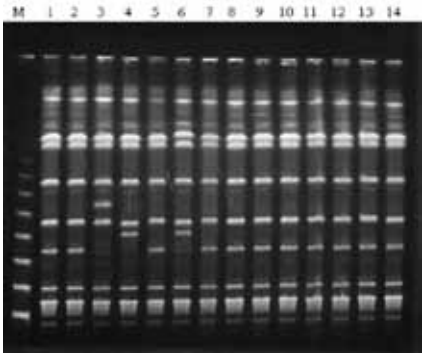


Fig.4. Macrorestriction pattern of *sma*I digested DNA of *S. agalactiae* isolated from bovine milk. Lane M: PFG Lambda marker, Lane 1-14: *S. agalactiae* field isolates of ST-483.

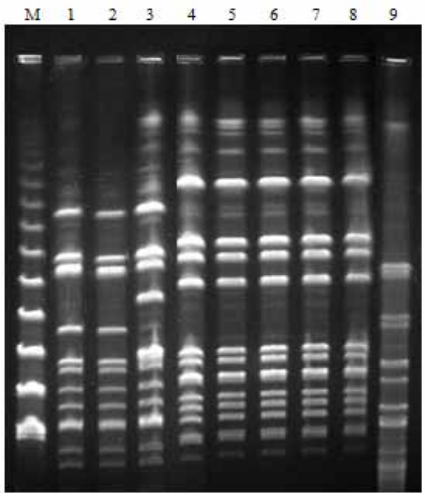


Fig.5. Macrorestriction pattern of *sma*I digested DNA of *S. uberis* isolated from bovine milk. Lane M: PFG Lambda marker, Lane 1-3: *S. uberis* isolates of ST-439, Lane 4-8: *S. uberis* isolates of ST-475 and Lane 9: *S. uberis* of ST-474.

S. aureus **-** PFGE analysis of selected *S. aureus* isolates covering different *spa* types revealed diverse PFGE banding patterns among the isolates. Related/identical patterns were found within and between *spa* types. Analysis of the pulsotypes using Gel compare II software revealed 85% similarity among the *S. aureus* isolates suggesting their closely related nature. A dendrogram derived using band-based similarity values using a cutoff of 85% (using Gel compare II), revealed two major clusters of isolates (Fig.6). The cluster I comprised of isolates of *spa* type t267, t359 and t6877 (PT 01, 02, 03, 04, 05, 06, 07, 08, 09, 10, 11, 12). Cluster II comprised of 71.11% of isolates and resolved into 27 pulsotypes (PTs 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40 and 41). From the cluster II, a single isolate with unassigned *spa* type was out grouped (PT 13). Strains T9W and BF82W of *spa* type t7680, isolated from different herds, showed similar band patterns demonstrating their genetic similarity. In contrast, few strains of different *spa* type isolated from different herds also showed similar band patterns (Fig.6). Similar to the *spa* phylogeny, PFGE analysis also out grouped the isolate with unassigned *spa* type. Moreover, there was a distinct clustering of isolates with *spa* type 267, 359 and 6877 reassuring the close relatedness of these clones.

Methicillin resistant CoNS-Diverse banding patterns (A, A_1, B, C and D) were observed among the 15 *mec* positive *S. haemolyticus* isolates with type V SCC*mec* element. Majority of the milk *S. haemolyticus* isolates (66.67%, 10/15) from herd I, revealed identical band pattern A, 6.67% (1/15) of isolate from milk illustrated closely related pulsotype with two band difference from pulsotype A, 20% (3/15) of isolates from milk, udder skin and teat cup liner had a distinct pattern (Fig.7). In case of human *S. haemolyticus*, isolates with SCC*mec* element non-typeable (*mec*C + no *ccr*) showed a unique pattern when compared to isolate with SCC*mec* non-typeable (*mec*B + no *ccr*). PFGE analysis of *mec* positive *S. epidermidis* isolates revealed that isolates with type V SCC*mec* element from herd II had identical pulsotypes where as milk isolate from herd III had a unique pattern. A diverse PFGE pattern was observed among the human *S. epidermidis* isolates with type V SCC*mec* element. Majority of the human *S. epidermidis* isolates (75%) with non-typeable SCC*mec* element (*mec*B +*ccr*C) had an identical pattern. Analysis of other *mec* positive CoNS viz. *S. sciuri*, *S. hominis* and *S. chromogenes* isolates revealed diverse pulsotypes. Further analyses of the pulsotypes to determine the relatedness of the isolates are underway.

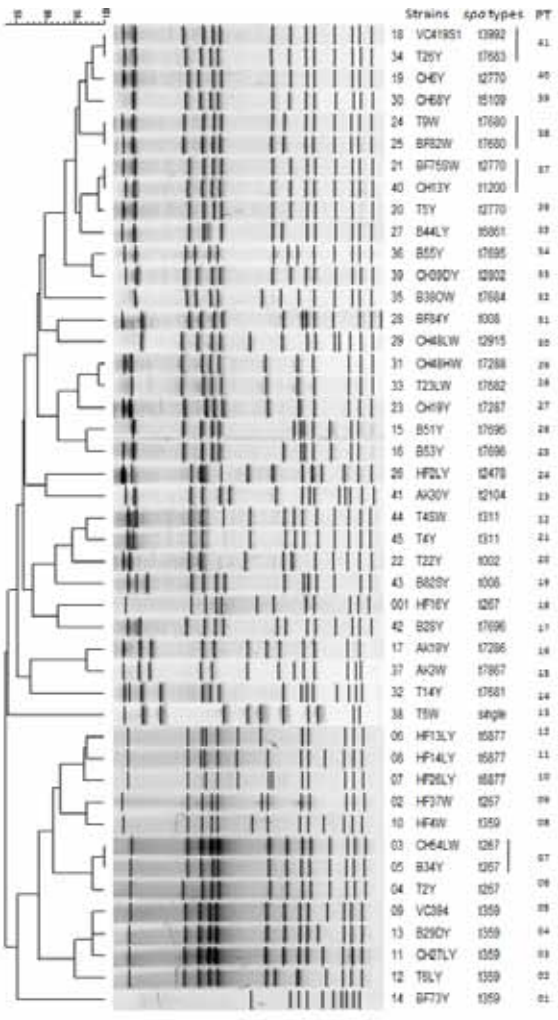


Fig.6. Dendrogram derived from UPGMA and band based similarities using Gelcompare II software, showing the levels of similarity among the *sma* I macrorestriction patterns of 45 strains of *S. aureus*. Columns to the right of the dendrogram shows the *S. aureus* strain name and number, *spa* types and pulsotype (PT).

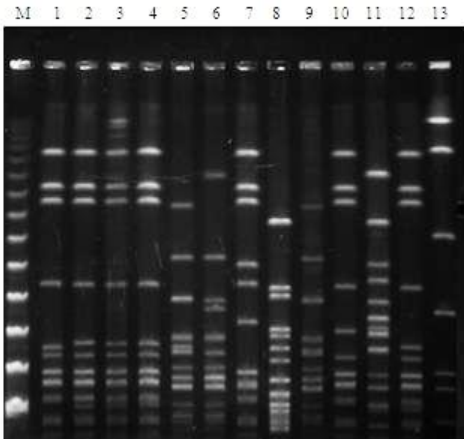


Fig.7. Representative Figure depicting the macrorestriction pattern of *sma*I digested DNA of *mec*A positive CoNS isolates. Lane M: PFG Lambda marker, Lane 1-4, 6: *S. haemolyticus* milk isolates, Lane 5: *S. haemolyticus* isolate from teat cup liner, Lane 7: *S. haemolyticus* isolate from udder skin surface, Lane 8: *S. chromogenes*, Lane 9-11: *S. sciuri* and Lane 12-13: *S. hominis*.

Quantitative multiplex PCR for detection of 14 mastitis pathogens

In this study, for detecting mastitis pathogens we constructed an IAC using housekeeping gene eEF-1a (Eukaryotic elongation factor 1-alpha) of *Oryza sativa*. Rice is one of the most important food crops and model monocot plant used for genetic and molecular studies. There is no possibility of encountering *Oryza sativa* in milk DNA. cDNA has been synthesized for the identified internal amplification control for the assay from plant. Its introduction will make sure the reliability of amplification and detection. IAC specific primer and probe are under synthesis.

Animal experimentation

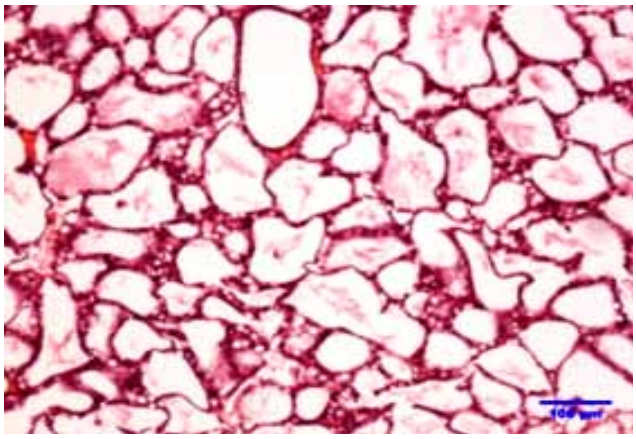
Seven prototype strains of Staphylococci (t6877, t267(2 strains)), *S. agalactiae* (ST-483), *S. uberis* (ST-439 and ST-475) and *E. coli* (ERIC group 7) were selected based upon their genotypes and virulence potential for experimental induction of IMI in Swiss-albino mice model. Of these, the experimental analysis of one strain each of *S. aureus* (t6877) and *S. agalactiae* (ST-483) were completed earlier. Evaluation of inflammatory cytokines at RNA transcript level and analysis of cellular changes by histopathological studies of the other experiments were taken up subsequently.

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

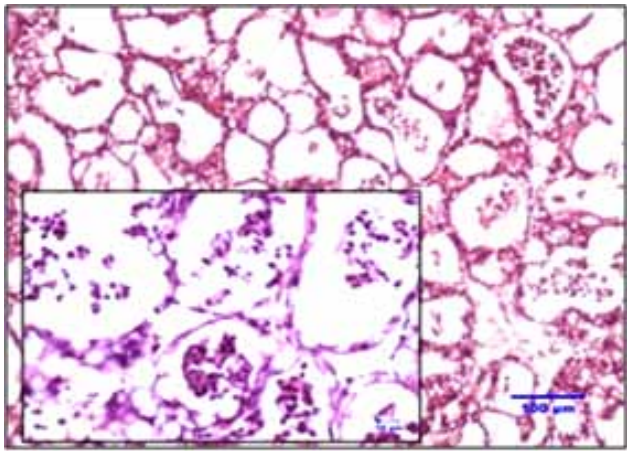
Escherichia coli

In PBS control, the alveolar lumen was 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.

At 4 hours post-infection, there was mild infiltration of alveolar macrophages in the alveolar lumen followed by mild infiltration of macrophages in to the lumen of alveoli at 8 hrs post infection.

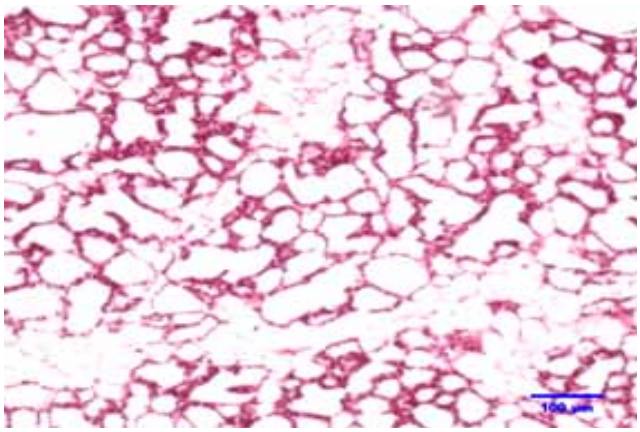


PBS

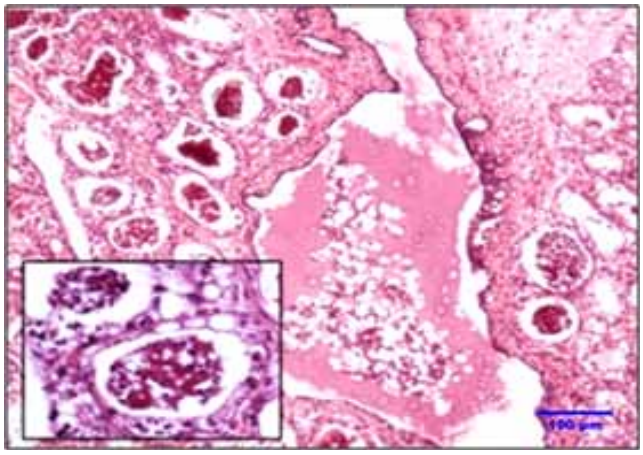


12 hours post infection

At 12 hrs post infection, there was a moderate infiltration of neutrophils and macrophages with damage to alveolar epithelium.

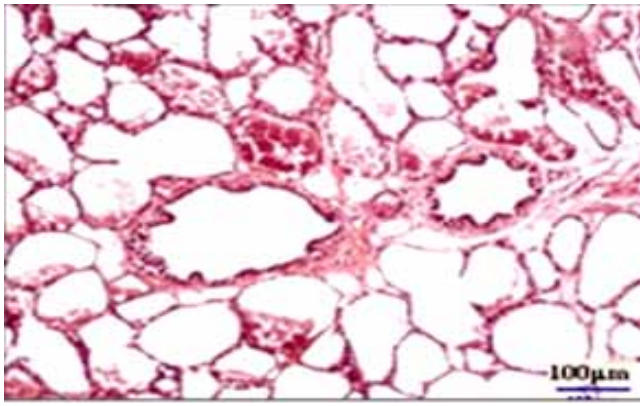


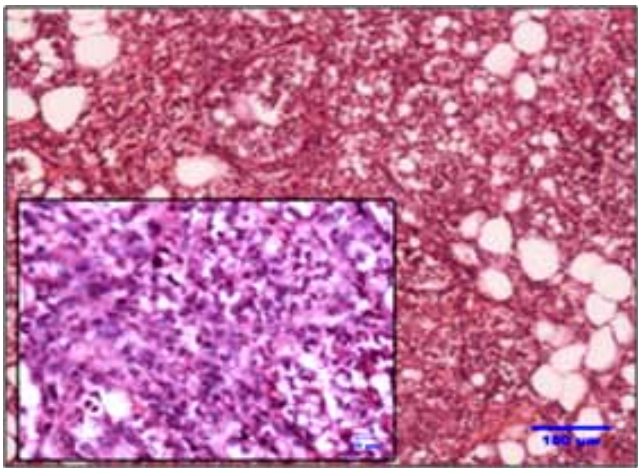
PBS



24 hours post infection

After 24 hrs, severe infiltration of polymorphonuclear and mononuclear inflammatory cells was seen in the alveolar lumen and also in interstitial space. Also necrosis of alveolar epithelial cells was observed.



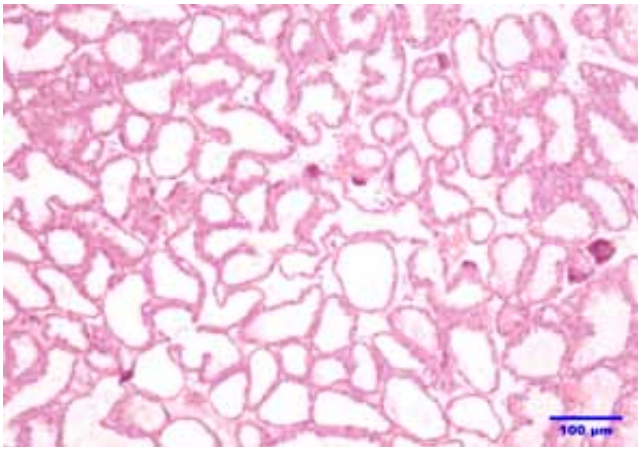


At 48 hrs of infection, there was complete loss of alveolar architecture due to severe necrosis and infiltration of predominantly mononuclear cells and less neutrophils.

***Staphylococcus aureus* (t267, HF16Y)**

On gross examination, the mammary glands showed congestion, redness from 24 hrs after inoculation. In PBS control, the alveoli were surrounded by few macrophages in the interlobular space which is normal.

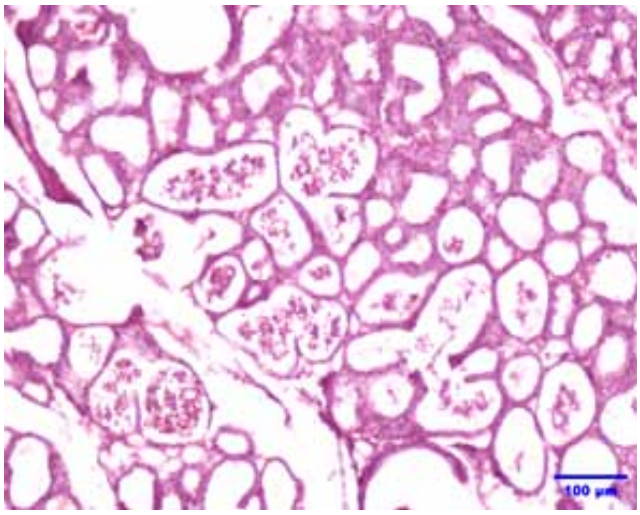
After 2, 4, 8 hrs of intramammary inoculation of *S. aureus*, the mammary gland tissue showed no apparent changes.



H&E, scaled to 100µm

12 hours post infection

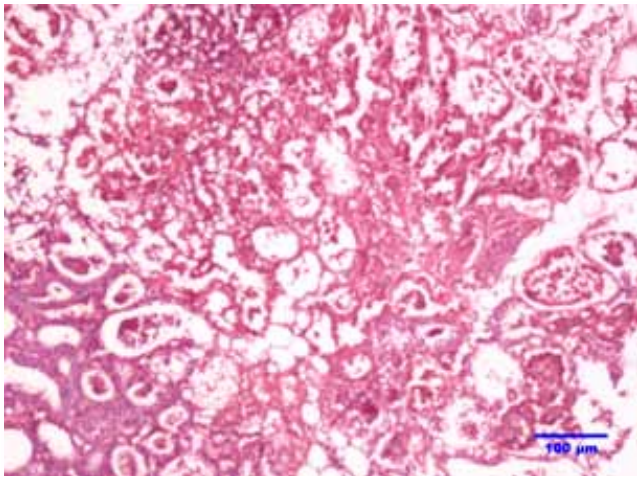
After 12 hrs of inoculation, mild infiltration of macrophages in the interstitial space with intact alveoli was observed.



H&E, scaled to 100µm

24 hours post infection

After 24 hrs of inoculation, infiltration of mononuclear cells into the lumen of alveoli. Loss of alveolar architecture and necrosis of alveolar epithelial cells were observed.



H&E, scaled to 100µm

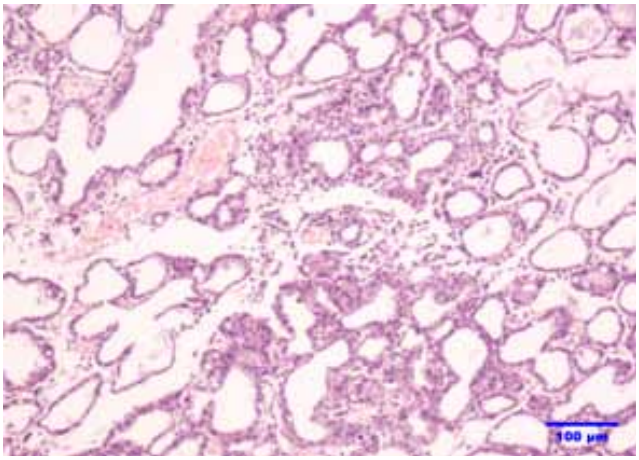
24 hours post infection

After 48 hrs, there was severe infiltration of mononuclear cells in the alveolar lumen and interalveolar space. The alveolar lumen filled with inflammatory cells and necrosis of the alveolar epithelial cells was observed

***Staphylococcus aureus* (t267, HF37W)**

S. aureus strain HF37W (t267) induced a similar but more intense cellular response than the other t267 strain. In PBS control, the alveoli were surrounded by few macrophages in the interlobular space which is normal.

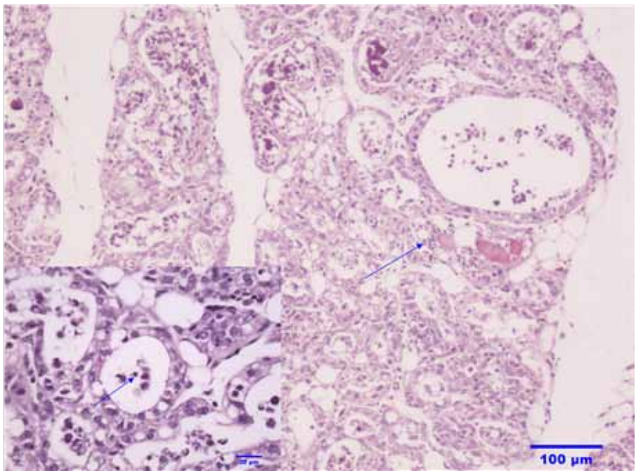
At 2, 4, 8 hrs of intramammary inoculation, the mammary gland showed no apparent changes. After 12 hrs of inoculation, moderate infiltration of inflammatory cells was observed in the interstitial space with intact alveoli.



H&E, scaled to 100µm

24 hours post infection

After 24 hrs of inoculation, severe infiltration of mononuclear cells into the lumen of alveoli. Necrosis of alveolar epithelial cells were observed



H&E, scaled to 100µm

48 hours post infection

After 48 hrs, there was damage of alveolar epithelial cells and severe infiltration of mononuclear cells in inter-alveolar space. Loss of luminal architecture of alveoli was observed.

Histopathological analysis of the two strains of *S. uberis* (ST-439 and ST-475) infected mammary tissues are underway.

Evaluation of selected inflammatory mediators at RNA transcript level in milk and tissue infected with *E. coli, S. aureus* and, *Streptococcus sp.* in time course manner

Many different mediators of inflammation are expressed at different times after pathogen or stimulus exposure. Cytokines are an important group of inflammatory mediators. Although cytokines play an essential role in the host response to infection, they can have deleterious effects on the host. Thus, there is a fine balance between the positive and negative effects of cytokines on the host that is dictated by the duration, amount, and location of their expression.

Quantitation of mRNA transcript level of genes associated with immune response mechanisms in mastitis induced by *S. aureus* (*spa* t6877) and *S. agalactiae* (ST-483) were carried out earlier by UPL based Real Time PCR. Subsequently, the relative quantification of mRNA levels 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 on intramammary challenge with *S. aureus* (*spa* t267, 2 strains), *S. uberis* (ST-439 and ST-475) and *E. coli* was carried out.

In the present study, *E. coli* challenge increased the mRNA abundance of the inflammatory cytokines *viz*., IL-2, IL-4, IL-6, IL-12, TNF-α, IFN-γ, GMCSF, TLR-11, IL-1β, RANTES, CXCl-5 showing peak response at 48th hour post infection (Fig.8). Amongst the members of TLR gene family screened, TLR4 which is known to be activated by lipopolysaccharide (LPS), a component of the outer envelope of Gram-negative bacteria (Ibeagha-Awemu etal 2008) was highly upregulated 2hrs post infection as assumed. *E. coli* endotoxin (lipopolysaccharide, LPS) when bound by host membrane proteins such as CD-14, causes release of proinflammatory cytokines recruiting neutrophils as an early, innate immune response. A significant 43.93 fold increase in levels of CD14 was noted at 24th hour post infection which followed by up-reguation of TNF-α and IL-1β, the two important pro-inflammatory cytokines, reaching its peak at 48th hour. The induced cytokines (IL-1 and IL-6) are known to be major and potent inducers for the production of acute-phase proteins in the liver.

Indeed marked induction of genes encoding acute-phase protein SAA3 was observed within the *E. coli* infected mammary gland showing a 1206 fold up-regulation of SAA3 at 24th hour post infection. However, whether the induction of acute-phase proteins in the mammary gland is an autocrine response of TLR4-mediated cytokine induction or a direct result of a TLR4-mediated signaling pathway remains unknown. The functions of the acute-phase proteins are not well described, but recent reports indicate role in leukocyte attraction (Zheng et al 2005). Elevated level of RANTES was observed in *E. coli* stimulated mammary gland after 24th hour than in *S. aureus* stimulated gland. This indicates a later role of RANTES in chemotaxis, important not for initiation but maintenance of inflammation. Neutrophil recruiting factor, CXCl-1, was strongly induced by the *E. coli* challenge showing 36.24 fold elevation at 24th hour and subsequently induced 45. 37 fold change of chemoattractant CXCl-5 known to recruit monocytes, eosinophils and basophils.

Strain variation was also noticed among the two t267 strains (Fig.9), one strain (HF37W) elicited a mixed immune response inducing elevation in the mRNA transcript levels of various genes at different time point whereas the other strain (HF16Y) induced abundance of mRNA transcripts of almost all the genes at 8h PI. The variation of the response according to the strain may reflect the earlier findings reported *in vivo* with some *S. aureus* strains that induced subclinical mastitis in contrast to others that induced mild to severe clinical mastitis (Haveri et al., 2005). A sharp decline in the mRNA levels of all genes was observed at 48th hour post infection. This short lived inflammatory response after *S.*

aureus infection could favour the establishment of *S. aureus* in the mammary gland. It has been postulated that persistent infection with *S. aureus* is associated with an impairment of the immune response mediated by factors of either host or *S. aureus* origin (Lahouassa et al., 2007).

Amongst the 18 genes studied, after intramammary challenge with *S. aureus* (t267), mRNA transcript levels of IL-2, IL-4, IL-6, IFN-γ, TNF-α TLR-9, TLR-11, TLR-12, CD14, CXCL-1 and CXCL-5 were elevated with peak intensity (4-8.4 folds) at 8th hour post infection followed by decline from 24th hour. Recognition of invading pathogens is the first deciding step in initiating immune defense. Although most studies describe TLR2 as a primary receptor for gram positive bacteria (Griesbeck et al., 2008), in the present study there was no significant change in TLR-2, instead TLR-4 showed 4.216 fold increase at 8th hour, taking a peak (4.9 folds) at 24th hour followed by a decline at 48th hour post challenge. Interestingly TLR9, 11 and 12 showed significant up regulation with maximum elevation of TLR 12 (7.082 fold) at 24th hour post infection. This pattern of TLR expression indicates better and strong pathogen recognition and emphasizes the importance of other members of TLR family apart from TLR2 and TLR4 as Pattern recognition receptors (PRR).

Cytokine production during *S. uberis* mastitis is not much documented. In the present study, two unique strains of *S. uberis* ST-439 (*sua*$^{+ve}$) and ST-475 (*sua* $^{-ve}$) were used for understanding the pattern of immune response associated with *S. uberis* IMI. This study provides insight into the inflammatory response during experimental *S. uberis* mastitis. Based on our findings, IMI with *S. uberis* elicited local production of cytokines, and peak concentrations of IL2, IL4, RANTES reached at fourth hour post infection. TLR 2 showed only 1.48 fold increase in mRNA transcript at the initial stage (2 hours post infection) and intensity did not increase with progression of time, indeed started declining from 12th hour onwards. Interestingly, TLR 11 and TLR 12 comparatively showed higher levels of mRNA with an increase of 2 fold at 2 hours, which increased gradually and peaked (4 fold) at 12th hour, followed by a decline at 24th hour. The major pro inflammatory cytokines showed a pattern of gradual increase with IL-1β taking a peak at 8th hour followed by TNF-α and IL-6 at 12th hour post infection. Neutrophil recruiting factor CXCl-1 was induced showing 5.166 fold increase in mRNA transcripts at 12th hour and parallely induced 4.984 fold increase of chemoattractant CXCl5 known to recruit monocytes, eosinophils and basophils. CXCl are intimately associated with inflammatory processes and are probably essential for the recruitment and activation of neutrophils into the infected mammary tissue.

S. uberis ST-475 (*sua* $^{-ve}$) infection induced the abundance of IL-6, RANTES, CXCl1, GMCSF, TNF-α, IL-12 and TLR 9 mRNA transcripts which were maximum at 24 hours post infection. Similar to the two t267 strains of *S. aureus*, strain specific differential immune response was found among the *S. uberis* isolates (Fig.10). An early and marked increase in the levels of pro-inflammatory cytokine IL-6 in both strains of *S. uberis* was observed at 4 hours post infection, whereas, the mRNA profile of the remaining inflammatory mediators showed marked difference between the two strains of *S. uberis.*

During bacterial infection of the bovine mammary gland, large numbers of leukocytes migrate into the udder, resulting in the establishment of a host response against the pathogen. The ability to recruit cells into the mammary gland during the bacterial growth phase is considered pivotal in curtailing bacterial pathogenesis. The faster the host can recruit effector cells, the earlier bacterial growth will be decelerated. Currently, the specific leukocyte populations mediating this immune response are not well defined. Most studies address the somatic cells and their distribution in the milk, but the leukocytes in the udder tissue could differ from those in the milk and may play a more important role in mammary immunity than the latter. Cell surface markers are used to identify the specific cell populations identified in the mammary immune response. There is an

increasing range of well-characterized monoclonal antibodies (mAbs) available for, and raised against, bovine cell surface markers. In the present study, the infiltration of immune cells especially lymphocyte subsets ($CD19^+$, $CD3^+CD4^+$, $CD3^+CD8^+$) to the mammary gland in response to intramammary infection was studied using cluster differentiation antigen specific monoclonal antibodies by flow cytometry. In a healthy udder tissue, the T-lymphocytes are predominantly CD8+ cells so that the $CD4^+$:$CD8^+$ ratio is less than unity (<1). We observed that as a result of *S. aureus* (*spa* t267) IMI, there was a proportionate increase of $CD4^+$ T lymphocytes which surpassed that of $CD8^+$ cells, thus altering the ratio of $CD4^+$:$CD8^+$ T lymphocyte to >1. In earlier studies, when the $CD4^+$ to $CD8^+$ ratio was examined in milk from healthy cows and cows with bacterial induced mastitis it was found that the $CD8^+$ T lymphocytes were predominant in milk from healthy animals while $CD4^+$ T lymphocytes were predominant in milk from animals with mastitis. $CD4^+$ T cells play an important role in the adaptive immune system and are essential in B cell antibody class switching, in the activation and growth of CD8+ T lymphocytes, and in maximizing bactericidal activity of phagocytes such as macrophages. Data on other strains are to be generated.

Bangalore Milk Union Limited (BAMUL) Project

Rajeswari Shome and M. Nagalingam

A total of 1624 milk samples were received from Bangalore urban and rural milk societies in the project. Milk samples were tested for anti-*Brucella* antibodies by milk ring test and 109 out of 1624 (6.71%) milk samples were positive. The highest positivity was recorded in milk samples received from Doddaballapur area and least prevalence in bulk milk coolers. Since the overall prevalence was found >5%, vaccination was suggested in the milk shed area of Bangalore urban and rural districts. The details of different milk routes are listed in Table 1.

Table 1. Details of different milk routes

S. No.	Milk Producers Co-op Society	No of samples received & tested	No. of positive samples	Percent positive
1	Hoskote	268	15	5.59%
2	Anekal	145	11	7.58%
3	Doddaballapur	201	21	10.44%
4	Doddaballapur BMC	96	15	15.62%
5	Vijipura	180	12	6.66%
6	Byrapatna	198	10	5.05%
7	Solur	296	Nil	Nil
8	Kanakapura	218	16	7.33%
9	Bulk milk coolers	318	9	2.83%
Total		**1624**	**109**	**6.71%**

Service Projects

Updating and Maintenance of Serum Bank

D. Hemadri, M. R. Gajendragad and S. S. Patil

Serum bank is a repository of serum which are catalogued, stored and preserved for any future use. In the serum bank, care is taken to preserve the immunological and other biochemical properties of the sera stored. Thus both the catalogue and the storage conditions are essential components of a successful serum bank.

In the serum bank, it is essential to fully document and identify each individual sample. For this it is compulsory to develop a database which contains all relevant information about the origin of the sample and test results obtained. Additional data that may be of interest, such as the animal's productivity, vaccination status may also be included. The serum bank catalogue should be well organised and maintained on a computer database with appropriate backup.

The terms of usage determine storage of the serum. For example, sera which are intended for periodic use need to be stored in multiple aliquots, while those intended for long-term storage may only need to be stored in two or three aliquots. Therefore it is essential to separate these two functions. Storage conditions determine quality of the serum over a period. Therefore care should be taken to minimise loss of immunological and other biochemical properties during the storage.

Development of MS Access based database (mini software)

Keeping the above things in mind, during the period under report it was envisaged to develop an access based database, which has all functions of mini software. As part of the development, an access based database has been designed and is currently undergoing various stages of testing and evaluation.

The database currently has seven forms (receipt entry, dispatch entry, results entry, result communication, storage, and queries).

Receipt entry form is used to enter information accompanied with the serum sample. It has 24 fields which are shown in the screen shot below. Many fields such as center, state, district, taluk, village etc. are given as drop down menus in order to prevent spelling errors as well as for faster entry of records.

Dispatch entry form is used for entering dispatch details to various labs. Fourteen fields, which are used for dispatch entry are shown in the screen shot below. Similar to entry form, the receipt form has many fields, which have dropdown menus.

Results entry form is used for entering the results of various laboratory tests. Barring the two fields (sender sample number, results date) remaining 11 fields are provided with drop down menus for faster data entry.

Results communication form is used for communicating the results to the sender. Upon entering the sender's reference number, the fields

such as ADMAS No, Sender's sample number, results of the tests etc are automatically shown in the field window.

Queries form is used for making various queries. Sample of queries form is given below. A click of a button generates answer/s to the queries shown below.

Storage form is used for recording the storage information on the serum samples received. It has provisions to record storage place besides the sample availability.

Besides the above forms, provisions have also been made to generate quarterly and date wise reports in the similar lines of queries form.

Report of the serum bank

During the period under report, a total of 4671 serum samples were received from various collaborating centers of AICRP as well as from other places. The details are given in the Table 1. The species-wise break up of samples is shown in the Pie Chart (Fig. 1). The serum bank arranged screening of these samples by aliquoting and distibuting one aliquot each to various laboratories. The remaining samples were aliquoted and catalogued and stored and preserved for any future use. Upon receipt of the results, the serum bank compiled and communicated screening results to the sender. Details of serum samples received (Table 1) and results of screening are given below (Table 2)

Table 1. Details of serum samples received.

Centre	SPECIES	Total
AHMEDABAD	Buffalo	59
	Cattle	167
	Goat	71
	Sheep	28
AHMEDABAD Total		325
ADRI, CUTTACK	Buffalo	11
	Cattle	126
	Goat	104
	Pig	5
	Sheep	5
ADRI, CUTTACK Total		251
CARI, A& N	Cattle	324
CARI, A& N Total		324
DIS, PUNE	Buffalo	75
	Cattle	105
	Goat	82
	Sheep	25
DIS, PUNE Total		287
IAH & VB, BANGALORE	Buffalo	5
	Cattle	5
IAH & VB, BANGALORE Total		10
IAH & VB, TRIVENDRU	Cattle	98
IAH & VB, TRIVENDRUM Total		98
SDAH, SRINAGAR	Cattle	823
	Goat	8
	Sheep	16
SDAH, SRINAGAR Total		847
SDDC, JAIPUR	Buffalo	121
	Camel	28
	Cattle	122
	Goat	129
	Pig	62
	Sheep	44
SDDC, JAIPUR Total		506
SDIL, BHOPAL	Buffalo	87
	Cattle	102
	Goat	568
	Pig	241
	Sheep	35
SDIL, BHOPAL Total		1033
SDLL, IMPHAL	Buffalo	22
	Cattle	332
	Goat	233
	Pig	39
	Sheep	24
SDLL, IMPHAL Total		650
VBRI, HYDERABAD	Buffalo	80
	Cattle	88
	Goat	84
	Pig	10
	Sheep	78
VBRI, HYDERABAD Total		340
Grand Total		4671

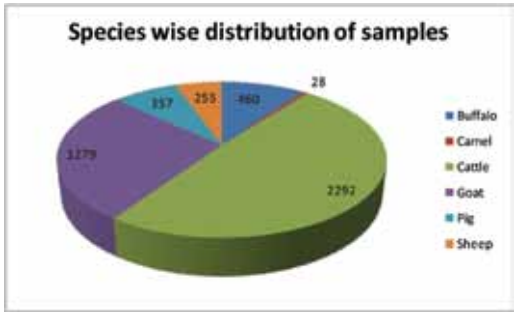


Fig. 1. Species wise distribution of serum samples

Table 2. Results of serum screening.

Centre	Bovine Brucellosis	Swine Brucellosis	IBR	CSF	Bov Bru+ IBR	Sui Bru+ CSF
ADRI, CUTTACK	4	NA	33	NA	3	NA
CARI, ANDAMAN & NICOBAR	33	NA	34	NA	5	NA
CVSC, AAU, GUWAHATI	0	NA	0	NA	0	NA
CVSC, GADVASU, LUDHIANA	0	NA	0	NA	0	NA
DIS, DAH, PUNE	30	NA	16	NA	1	NA
FMD TYPING SCHEME, AHMEDABAD	29	NA	78	NA	18	NA
IAH & VB, BANGALORE	2	NA	0	NA	0	NA
IAH & VB, THIRUVANATHAPURAM	5	NA	0	NA	0	NA
SDAH, SRINAGAR	40	NA	56	NA	11	NA
SDIL, BHOPAL	10	21	25	69	6	26
SDLL, DV & AHS, IMPHAL	89	9	27	3	52	0
SDDC, JAIPUR	0	1	0	32	0	0
VBRI, HYDERABAD	14	0	29	0	34	0
Total	256	31	298	104	130	26

The numbers within the column indicate number of samples tested positive against the column header. NA: Not Applicable

Sero-epidemiology of bovine brucellosis

Rajeswari Shome and M. Nagalingam

Brucellosis is a bacterial disease caused by various Brucella species, which mainly infect cattle, swine, goats, sheep and dogs. Humans generally acquire the disease through direct contact with infected animals. Person-to-person transmission is rare. The disease causes flu-like symptoms, including fever, weakness, malaise and weight loss in humans. The sero-epidemiology on Brucellosis, which was initiated more than decade ago is still continuing and ensuing paragraphs highlights the work done during 2011-12.

A total of 2912 random sera samples from cattle (1056), buffaloes (332), sheep (180), goats (828) and swine (307) were received from 10 AICRP centers for brucellosis screening during the period. 363 out of 2915 (12.46%) samples were found positive by ELISA. Among five livestock species tested, highest seropositivity was found in swine (18%) followed by cattle and buffaloes (13%) and lowest prevalence of brucellosis was recorded in sheep and goats (≈8%).When state wise prevalence rate was compared, highest prevalence was recorded in the samples of Orissa and Andhra Pradesh (21%); followed by Gujarat (15%). Rajasthan and Jammu & Kashmir states had almost 12% prevalence rate. Similarly 11% prevalence was recorded in states of Madhya Pradesh and Maharashtra, whereas only 3 out of 10 samples received from Karnataka were positive. Least prevalence of brucellosis was recorded in Manipur (7%) and Kerala (5%) states. The species and state wise sample results are presented in Table 1 and Figs 1 & 2.

- During the period, 473 sera samples were received from five institutions for diagnosis of brucellosis and 42 out of 473 (8.8%) were declared as seropositive and reports were communicated. Interestingly, 17 camel samples were tested using Protein-G based ELISA and the results were found satisfactory when compared with conventional serological tests. The sample and result details are given in Table 2.

- Brucellosis Lateral flow test strips developed by TANUVAS, Chennai ; MRT and RBPT antigens manufactured by VBRI, Hyderabad and Indirect ELISA kit for the detection of *M. paraTB* antibodies developed by CIRG, Magdhoom were evaluated using the standard positive and negative test, 20 field sera and milk samples and validation results were communicated.

Table 1. Brucellosis screening results of sera samples received from AICRP Centers. Numbers in the parenthesis indicate the number of positive samples.

S.No	State	Bovine	Cattle	Buffalo	Sheep	Goat	Swine	Total	Percent Positive
1	Rajasthan	-	48 (14)	52 (5)	24 (2)	60 (5)	41 (1)	**225 (27)**	**12**
2	Manipur	-	133 (5)	21 (6)	13 (0)	67 (5)	39 (5)	**273 (21)**	**7.69**
3	Maharashtra	-	103 (20)	75 (9)	25 (1)	82 (4)	-	**285 (34)**	**11.92**
4	Gujarat	94 (29)	73 (12)	59 (7)	28 (0)	71 (2)	-	**325 (50)**	**15.38**
5	Karnataka	-	5 (3)	5 (0)	-	-	-	**10 (3)**	**30**
6	Kerala	-	94 (5)	-	-	-	-	**94 (5)**	**5.31**
7	Jammu & Kashmir	447 (55)	-	-	-	-	-	**447 (55)**	**12.3**
8	Madhya Pradesh	-	102 (11)	85 (6)	24 (0)	494 (40)	227 (52)	**932 (109)**	**11.69**
9	Andhra Pradesh	-	35 (5)	35 (11)	53 (13)	54 (9)	-	**177 (38)**	**21.46**
10	Orissa	-	131 (21)		13 (0)	-	-	**144 (21)**	**14.58**
Total		**541 (84)**	**724 (96)**	**332 (44)**	**180 (16)**	**828 (65)**	**307 (58)**	**2912 (363)**	**12.46**
Percent positive		**15.52**	**13.25**	**13.53**	**8.88**	**7.85**	**18.89**	**12.46**	

Table 2. Brucellosis screening results of the samples received from other Institutions

S. No.	Place	Species	No. of samples screened	Positives
1	Private farm, Karnataka	Cattle	226	52
2	Dept .of AH & VS, Meghalaya	Cattle	35	5
3	NDRI, Bangalore	Cattle	159	2
4	NRC Camel, Bikaner	Camel	17	1
5	AAU Farm, Assam	Goat	36	2
Total			**473**	**42 (8.8%)**

Seroepidemiology of Infectious bovine rhinotracheitis (IBR) in Indian bovines

S. S. Patil, D. Hemadri and H. Rahman

Serum repository of the institute gets random stratified sera samples of bovines in addition to other species from all the coordinating units (CU) yearly. CUs located in Punjab, West Bengal, Asom, Meghalaya, Jharkhand did not contribute any sera samples and in turn Andaman and Nicobar Islands have sent 324 sera samples of bovine origin. A total of 2275 sera samples of bovine origin from 11 states were screened for IBR antibodies and 507 sera samples were found positive. The highest prevalence was 48.7% (75/154) in Manipur and lowest was 2.58% in Karnataka (4/141). The variation in positive prevalence was due to sample size and not following the randomization techniques in collection of samples.

State wise seroprevalence of IBR during 2011-12

Sl No	State/Union Territory	No Tested	No Positive	Positive percentage
1	Jammu & Kashmir	447	68	15.2
2	Andhra Pradesh	141	74	52.4
3	Karnataka	155	4	2.58
4	Odisha	271	54	20
5	Kerala	90	0	0
6	Andaman& Nicobar	324	39	12
7	Manipur	154	75	48.7
8	Maharashtra	180	17	9.4
9	Gujarat	226	97	42.9
10	Madhya Pradesh	187	29	15.5
11	Rajasthan	100	50	50
		2275	507	22.5

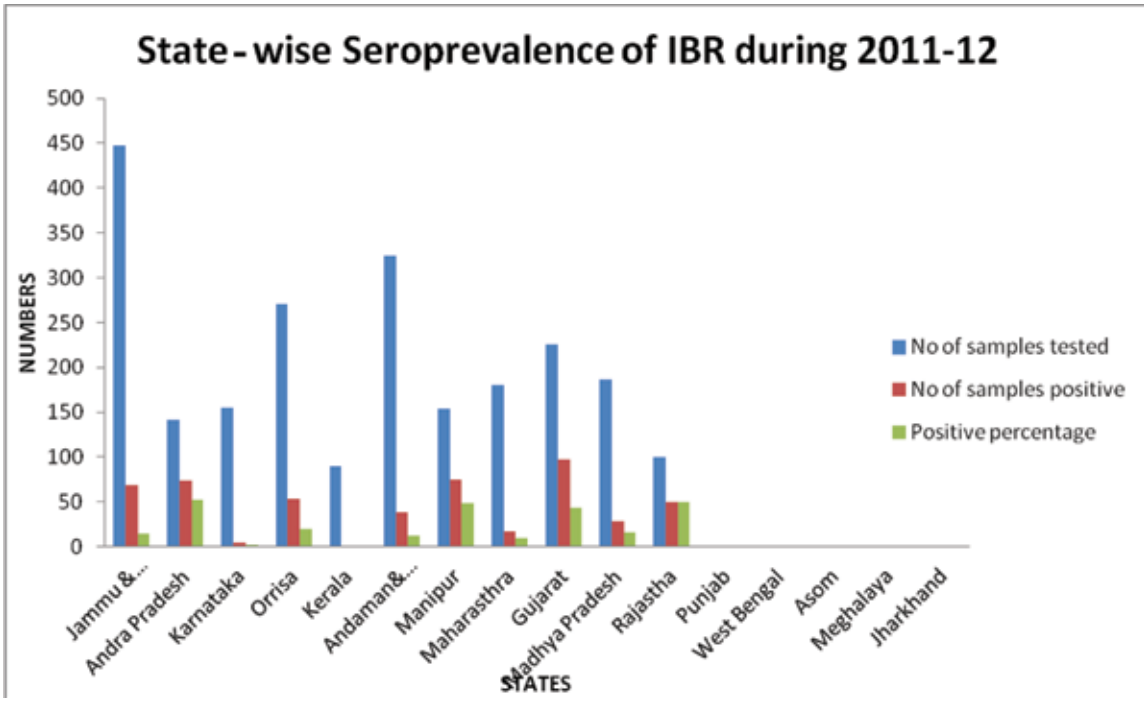
Cumulative Seroprevalence of IBR

The overall seroprevalence of IBR during 1995-2011 was found to be 35.85 % (Table 1, Fig 1) In cumulative study 59284 serum samples from different parts of the country were tested by AB-ELISA during these years and 21256 samples were found positive. The variation in the overall prevalence of IBR may be attributed to the sample size.

Table 1. Year-wise cumulative report on seroprevalence of IBR during 1995-2011

S. No.	Year	No. Tested	No. positive	Apparent % positive
1.	1995-96	3428	1303	38
2.	1996-97	3521	1096	31
3.	1997-98	1442	599	42
4.	1998-99	1675	767	46
5.	1999-01	6883	2776	40
6.	2001-02	3373	785	23
7.	2002-03	7933	3271	41
8.	2003-04	1300	668	51
9.	2004-06	9564	3507	37
10.	2006-07	2820	1197	42
11.	2007-08	4270	1242	29

S. No.	Year	No. Tested	No. positive	Apparent % positive
12	2008-09	4821	1423	30
13	2009-10	4496	1494	34
14	2010-2011	1483	621	42
15	2011-12	2275	507	22.5
	TOTAL	59284	21256	35.85



Miscellaneous

Quinquennial Review Meet (QRT)

The Third QRT of the PD_ADMAS was held during the 2011-12. The team conducted a series of meeting under the chairmanship of Dr. A. T. Sherikar, The QRT took the note of the salient achievements of PD_ADMAS during the period under review (2007-11) and the research work on epidemiology of important livestock diseases and diagnostics. The teawere of the opinion that the research work conducted at the institute, in general, is in tune with the mandates of the institute. The team appreciated the work on the time series analysis of major diseases of livestock, the spatial epidemiology of HS, BQ, PPR, ET, CSF, BT and the molecular epidemiology of Leptospira, BT and CSF, population epidemiology of IBR, Brucellosis, development and standardization of techniques for detection of carrier status of Surra, antibodies to Brucella organisms in small ruminants and pigs and CSFV in pigs. The team also hailed the compilation of the livestock diseases of the country and its analysis to assess the trend of the diseases and plotting of eco-pathozones of various diseases. The team suggested various measures to overcome constraints faced by the institute.

Research Advisory Committee Meet (RAC)

The meeting of the Research Advisory Committee of PD_ADMAS was held on 8th July 2011. The committee under the chairmanship of Dr R. N. Sreenivas Gowda thoroughly discussed the research projects taken up at PD_ADMAS and made few recommendations. During the meeting four new reasearch projects were approved.

Patents

Granted

Patent for Indirect-ELISA for sero-screening of brucellosis in sheep and goat by R. Shome et al., (patent application filed vide No. 01592/CHE/2008) has been granted on 20.01.2012 - Patent No 250709.

Applied

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Taqman Real Time PCR based kit for detection of BoHV-1 in clinical samples by B. M. Chandranaik, D. Rathnamma, S.S. Patil, S. Isloor and K.P.B. Ramesh. 778/CHE/2012 Dated 01/03/2012

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Patil SS, Hemadri D, Rahman, H (2011). Scenario of Classical swine fever in Indian Pig Population. In National seminar on Status and Scope of Pig Farming in India- An Appraisal 17-18 November, 2011, ARS, Kattupakkam, Chennai.

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

SI. No.	Name of the seminar/ workshop/ Training	Venue	Date
1.	1st Meeting of 3rd QRT Meeting	PD_ADMAS, Bangalore	19th & 20th May 2011
2.	Interactive Meeting of Stake holders	PD_ADMAS, Bangalore	25th May 2011.
3.	Foundation day of PD_ADMAS	PD_ADMAS, Bangalore	1st July 2011
4.	IMC Meeting/IRC of PD_ADMAS	PD_ADMAS, Bangalore	7th July 2011
5.	RAC Meeting of PD_ADMAS	PD_ADMAS, Bangalore	8th July 2011
6.	FAO Training programme on Introduction to Spatial Epidemiology for Scientists of PD_ADMAS	PD_ADMAS, Bangalore	2nd -5th August 2011
7.	Interactive Meeting with officials of NDDB for construction of administrative building and BSL II laboratory	PD_ADMAS, Bangalore	8th -9th August 2011
8.	2nd Meeting of 3rd QRT Meeting	PD_ADMAS, Bangalore	20th August 2011
9.	Model Training Programme on "Brucellosis Control in India"	PD_ADMAS, Bangalore	5th -12th September 2011
10.	Hindi Week	PD_ADMAS, Bangalore	14th – 21st September 2011
11.	3rd meeting of 3rd QRT Meeting	PD_ADMAS, Bangalore	23rd September 2011
12.	CIC & CAC of NAIP	PD_ADMAS, Bangalore	18th October 2011
13.	Institutional Animal Ethics Committee meeting	PD_ADMAS, Bangalore	22nd October 2011
14.	Interactive Meeting with officials of NDDB for construction of administrative building and BSL II laboratory	PD_ADMAS, Bangalore	5th November 2011
15.	19th Annual Review Meet of AICRP_ADMAS	Jaipur	8th December 2011
16.	Project Implementation and Technical committee meeting for construction of administrative building and BSL II laboratory	New Delhi	17th December 2011
17.	Bangalore milk union limited (BAMUL) project , one day project concluding meeting	PD_ADMAS, Bangalore	17th December 2011

SI. No.	Name of the seminar/ workshop/ Training	Venue	Date
18.	Interactive meeting with NIC, NADRS	PD_ADMAS, Bangalore	28th December 2011.
19.	Interactive meeting with NIC, NADRS	PD_ADMAS, Bangalore	3rd January 2012.
20.	Interactive meeting with AWAKE, NGO to discuss the implementation of TSP in Tribal area	PD_ADMAS, Bangalore	4th January 2012
21.	Training cum workshop on "Animal Disease Informatics" to all the AICRP_ADMAS Collaborating units	PD_ADMAS, Bangalore	28th Feb to 5th March 2012
22.	One day sensitization training program on brucellosis control under DADF sponsored *National control program on brucellosis (NCBP)* was organized	PD_ADMAS, Bangalore	30.03.2012.

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

S. No.	Name of the seminar/workshop/ Training	Venue	Date	Scientist Attended
1	Annual Review Meeting of OPZD-project	New Delhi	May 16, 2011.	Dr. V. Balamurugan
2	Annual Review Meeting of AINP-BT	NewDelhi	May 16, 2011.	Dr. D. Hemadri
3	Zoonotic Meeting organized by NCDC	Directorate of Health Services, New Delhi	May 31, 2011.	Dr. H. Rahman
4	FAO Consultative Workshop on Regional Epidemiology and Laboratory Networking in the SAARC Region	Kathmandu, Nepal.	July 27-29, 2012.	Dr. M. R. Gajendragad
5	Training programme on "DATA analysis using SAS" under the National Agricultural Innovation Project (NAIP) Consortium "Strengthing statistical computing for NARS" funded by NAIP	Department of Agricultural Statistics, UAS, GKVK, Bangalore	Aug 8-13, 2011.	Dr. V. Balamurugan
6	Workshop on "Rinderpest Eradication in India"	ICAR, New Delhi	August 23, 2011.	Dr. H. Rahman
7	Workshop on "Classical swine fever" of NEH Region	AAU, Guwahati	August 24-25, 2011.	Dr. H. Rahman

S. No.	Name of the seminar/workshop/ Training	Venue	Date	Scientist Attended
8	National stakeholder consultation on climate change platform	CRIDA, Hyderabad	September 19-20, 2011.	Dr. B. R. Shome Dr. S. S. Patil
9	Participated training on "Bovine TB diagnostics"	Indian Immunologicals Ltd, Hyderabad	October 4-8, 2011.	Dr. V. Balamurugan
10	II meeting of Nano Technology Flatform	CIFE, Mumbai	October 7-9, 2011.	Dr. D. Hemadri
11	Interactive meet with Hon'able Agriculture Minister, Government of India	NASC, New Delhi.	November 7, 2011 .	Dr. D. Hemadri
12	National conference of Indian Academy of Tropical Parasitology conducted by Government Medical College and Hospital	Nagpur	November 11-13, 2011.	Dr. P. P. Sengupta Dr. P. Krishnamoorthy
13	III meeting of Nano Technology Flatform	TANUVAS, Coimbatore	November 10-11, 2011.	Dr. D. Hemadri
14	National seminar on "Status and Scope of Pig Farming in India- An Appraisal"	ARS, Kattupakkam, Chennai	November 17-18, 2011.	Dr. S. S. Patil
15	Five day training programme on Laboratory Engineering and Equipment Maintenance at CSIRO, Australian Animal Health Laboratory	Geelong, Australia	November 21-25, 2011.	Dr. H. Rahman
16	XIX Annual Review Meet of PD_ ADMAS	Jaipur	December 8, 2011.	Dr. H. Rahman Dr. M. R. Gajendragad Dr. B. R. Shome Dr. D. Hemadri Dr. S. S. Patil
17	Workshop cum training programme on "Software installation" under "Strengthening of statisticalcomputing for NARS"	University of Agricultural Sciences, GKVK, Bangalore	December 13-14, 2011.	Dr. P. Krishnamoorthy
18	VIROCON 2011	NRCE, Hisar	December 29-31, 2011.	Dr. D. Hemadri
19	National Symposium on "Innovative research approaches for diagnostic pathology"	Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai	December 29-30, 2011.	Dr. P. Krishnamoorthy

S. No.	Name of the seminar/workshop/ Training	Venue	Date	Scientist Attended
20	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"	Department of Veterinary microbiology, Veterinary College and Research Institute, Namakkal		Dr. M. Nagalingam
21	Brain storming session on "Strategies for propagation and augmenting productivity of Mithun in NEH Region"	NRC for Mithun, Dimapur, Nagaland	January 12-14, 2012.	Dr. M. R. Gajendragad
22	FAO Sponsored International Training/Workshop on TAD info and spatial epidemiology organized by FAO at FAO Regional office for Asian and Pacific region in Bangkok	Bangkok, Thailand	January 24-February 3, 2012.	Dr. D. Hemadri Dr. V. Balamurugan
23	Valedictory programme on Disease Informatics	CIFE, Mumbai	Jan 30, 2012.	Dr. H. Rahman
24	Two days workshop on "Microarray and Next Generation Sequencing Data Analysis"	Biokart India (P) Limited in Collaboration with Bionivid technology Ltd. Bangalore	January 27-28, 2012.	Dr. B. R. Shome
25	FAO-ICAR International Conference on Scientific developments and technical challenges in the progressive control of foot and mouth disease in South Asia.	NASC, New Delhi	13-15 February, 2012.	Dr. H. Rahman Dr. D. Hemadri Dr. S. S. Patil Dr. B. Ganesh Kumar
26	IV meeting of Nano Technology Flatform	NASC, New Delhi	March 12, 2012.	Dr. D. Hemadri
27	ICAR-NAE short training on "Molecular techniques in diagnosis and prophylaxis of diseases of farm animals and poultry"	Division of Pathology, Indian Veterinary Research Institute, Izatnagar	March 12-17, 2012.	Dr. P. Krishnamoorthy
28	DBT network-workshop on Development of Brucellosis Translational Research Programme	PD_ADMAS, Bangalore	March 20-21, 2012.	Dr. H. Rahman Dr. B. R. Shome Dr. R. Shome Dr. V. Balamurugan Dr. M. Nagalingam

Awards / Fellowsips / Recognition

DBT CREST fellowship Award for 2010-11 to Dr. H. Rahman.

Dr. H. Rahaman, Project Director has been nominated as Chairman, High Level Expert Committee on Highly Pathogenic Avian Influenza, DADF, New Delhi

Dr. H. Rahaman, Project Director has been nominated as Member, Board of Management, Indian Veterinary Research Institute, Izatnagar, Bareilly, U.P.

Dr. H. Rahaman, Project Director has been nominated as Member, Board of Management, Kerala Veterinary and Animal Science University, Thiruvananthapuram

Dr. H. Rahaman, Project Director has been nominated as Member, Research Advisory Committee, ICAR Research Complex for Goa

Best Research Paper Presentation Award for "Molecular Epidemiology of Bovine Herpesvirus - 1 and Development of TaqMan Real Time PCR based Kit for detection of BoHV-1 in Clinical samples by B.M. Chandranaik, D. Rathnamma, S.S. Patil and C. Renukaprasad at *International Conference and XXV Annual Convention of Indian Association of Veterinary Microbiologist, Immunologists and Specialists in Infectious Diseases [IAVMI]*, 9-11 June, 2011, Veterinary College, Bangalore.

Best Poster Presentation Award for "Emergence of Coagulase negative Staphylococcus in subclinical bovine mastitis in India" by N. Krithiga, D. Velu, M. Bhuvana, S. Das Mitra, A. Banerjee, Jagadish H, R. Shome, S. Isloor, K. Prabhudas, Shome BR at *International Conference and XXV Annual Convention of Indian Association of Veterinary Microbiologist, Immunologists and Specialists in Infectious Diseases [IAVMI]*, 9-11 June, 2011, Veterinary College, Bangalore.

Best Poster Presentation Award for the research topic entitled Antigen and antibody kinetics in PPR virus infected and vaccinated goats by A. Patel, K. K. Rajak, V. Balamurugan, A. Sen, S.B. Sudhakar, V. Bhanuprakash, R.K.Singh and A.B. Pandey at *International Conference and XXV Annual Convention of Indian Association of Veterinary Microbiologist, Immunologists and Specialists in Infectious Diseases [IAVMI]*, 9-11 June, 2011, Veterinary College, Bangalore.

Best Poster Presentation Award for poster presented to D. T. Pal, N.K.S. Gowda, Swati Verma, G. Maya, A. Raghavendra, N.C. Vallesha, P. Krishnamoorthy, K. T. Sampath. (2011). Study on chelated copper and zinc on tissue utilization and immunity in rats. In 14th Biennial animal nutrition conference on *Livestock productivity and enhancement with available feed resources.* 3-5 November 2011, College of Veterinary and Animal sciences, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India.

Dr. V. Balamurugan has been nominated as Sectional Editor (Veterinary virology) of *Indian Journal of Virology* published by Springer.

Dr. D. Hemadri has been nominated as Editor of *Dataset papers in Microbiology* published by Hindawi.

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. R.N. Sreenivasa Gowda, Former VC, KVAFSU, Bengaluru
Dr. M. Rajasekhar, Former Project Director, PD_ADMAS
Dr. B. Pattnaik, Project Director, PDFMD
Dr. Leo Loth, FAO, Delhi
Dr. Paul White, FAO, Delhi
Dr. M. Moni, Deputy Director General, NIC, New Delhi
Dr. A. B. Negi, National Project Co-ordinator, FAO, New Delhi
Dr. John Weaver, Team Leader / Chief Technical Advisor, ECTAD, India
Dr. P. C. Mahanta, Director, DCWFR, Bhimatal
Dr. Abdur Rahaman, President, Commonwealth Vety Association, India

Important Committees

Institute Management Committee

Name	Designation
Dr. H. Rahman	Chairman
Dr. Gaya Prasad	Member
Dr. R. Venkataramanan	Member
Dr. S. Kulkarni	Member
Dr. M. R. Gajendragad	Member
Mr. P. N. M. Nair	Member Secretary

Research Advisory Committee

Name	Designation
Dr. R. N. Srinivasa Gowda	Chairman
Prof. K. Kumanan	Member
Dr. M. L. Mehrotra	Member
Dr. B. Pattnaik	Member
Dr. M. Rajasekhar	Member
Dr. S. N. Singh	Member
Dr. H. Rahman	Member
Dr. Gaya Prasad	Member
Dr. M. R. Gajendragad	Member Secretary

Quinquennial Review Team

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

Staff Position during 2011-12

S. No.	Name	Designation	Period from
1.	Dr. H. Rahman	Project Director	April, 2011
Scientific Staff			
1.	Dr. M. R. Gajendragad	Principal Scientist	Feb, 2006
2.	Dr. B. R. Shome	Principal Scientist	May, 2006
3.	Dr. Divakar 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. B. Ganesh Kumar	Senior Scientist	Feb, 2012
7.	Dr. V. Balamurugan	Senior Scientist	Jun, 2009
8.	Dr. S. S. Patil	Scientist	Feb, 2006
9.	Dr. G. Govindaraj	Scientist	Nov, 2011
10.	Dr. P. Krishnamoorthy	Scientist	Jan, 2008
11.	Dr. Mohd Mudassar Chanda	Scientist	Jun 2009
12.	Dr. Jagadish Hiremath	Scientist	Aug, 2009
13.	Dr. M. Nagalingam	Scientist	Aug, 2009
Administrative Staff			
1.	Mr. P. N. M. Nair	Admn Officer	May, 2011
2.	Mr. S. R. Nataraj	Asst Admn Officer	Feb, 2007
3.	Mr. R. K. Babu	AF&AO	May, 2011
4.	Mr. Rajeevalochana	Assistant	Apr, 2007
5.	Mr. N. Narayanaswamy	Assistant	May, 2002
6.	Mr. M. Lakshmiah	Assistant	Jul, 2011
7.	Mrs. Uma. S.	PA	Jun, 2011
8.	Mrs. Saranya	Stenographer	Oct, 2011
9.	Ms. Sridevi. G. C.	LDC	Nov, 2011
10.	Ms.Rekha Priyadarshini	LDC	Nov, 2011
11.	Mr.Gangadareshwara.L.	LDC	Nov, 2011
Supporting Staff			
1.	Mr. M. K. Ramu	SSS	Aug, 2007
2.	Mr. H. Shivaramiah	SSS	Sep, 2007
3.	Mr. B. Hanumantharaju	SSS	Sep, 2007

Resource Generation

S. No.	Type Activity	Units sold	Amount (in Rs)
1	Sale of IBR ELISAkits	14	1,50,000
2	Sale of *Brucella* AB-ELISA kits	06	75,000
3	Sale of *Brucella* I-ELISA kits (Sheep & Goat)	03	37,500
4	Sale of *Leptospira* staining kit	01	900
5	Bench fee received for PG Research/Training	-	28,000
	Total		2,91,400

Budget 2011-12

Head of Account		Non Plan Rs (In Lakh)		Plan Rs (In Lakh)	
		Allocation	Expenditure	Allocation	Expenditure
Recurring Expenditure	Establishment Charges	203	201.97		
	Travelling Allowance	0.5	0.44	40.72	39.02
	HRD	0	0	2.5	1.19
	Contingencies	54.1	49.91	143.48	122.37
	TSP	0	0	140	134.44
	Sub Total (A)	257.6	252.32	326.7	297.02
Non-recurring	Equipments			73.3	67.09
	Works			1240	1240
	Vehicles				
	Furniture	3	2.36		
	Sub Total (B)	3.00	2.36	1313.30	1307.09
	Grand Total (A+B)	**260.60**	**254.68**	**1640.00**	**1604.11**
Revenue receipts including TDR interest					Rs.15.40
Interest earned on loans/advances					Rs.0.47
Miscellaneous					Rs.0.63

ADMAS News

Institute's Activities

All India Coordinated Research Project on Animal Disease Monitoring and Surveillance (AICRP on ADMAS)

AICRP of PD_ADMAS has 15 Collaborating Units (CU) located across the country for effective analyses of economically important livestock diseases. Of 15 CUs, two are located in Veterinary Colleges, one in ICAR institute and remaining 12 CUs are located in the State Disease Diagnostic Centers. The livestock disease reporting from CUs of AICRP is being stored in the database of the Directorate for further analyses. The constant efforts are made to procure quality data from different parts of the country and presently, the database has 90,104 records related to livestock diseases.

In order to have more quality data and true representation at national level, 16 more Collaborating Units in different parts of the country including one at the Directorate is proposed in the XII five year plan.

All the CUs will be actively engaged in sero-monitoring of important livestock diseases based on sample frame, investigation of endemic and emerging livestock disease outbreaks in respective area using innovative technologies, participation/strengthening of national livestock serum bank , 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.

Existing coordinating unit Newly proposed coordinating unit

Tribal Sub Plan (TSP)

Tribal Sub-Plan (TSP) is a strategic policy plan to look for overall development of scheduled tribes of India. The plan aims to bring the tribal population at par with other sections of the society and protect them from exploitations. Though TSP was started long back, it got strengthened in the eleventh five year plan with major objective is "to reform TSP and restore its dynamic character to make it an effective instrument for tribal development".

As per the guidelines of the planning commission, ICAR created a Tribal Sub-Plan Cell at its head quarter on 21st March, 2011 with a clear objective to bridge the gap in socio-economic development of the scheduled tribes. ICAR issued guidelines to implement the plan in agriculture, animal husbandry and fisheries related developmental works in Tribal areas. Accordingly, a Nodal Officer was nominated at this Directorate to implement the TSP through Collaborating Units (CU) of All India Coordinated Research Project (AICRP) on Animal Disease Monitoring and Surveillance (ADMAS). Based on the ratio of the tribal population against the total population of different states of the country, budget distribution was made and north eastern states were given additional funds to cover the remote places of the tribes. The CUs were supplied with guidelines to implement TSP and instructed to select such villages wherein the tribal population should be more than 60% of the total population of villages. Punjab state did not have any tribal population and so was not included in the plan. Taking keen interest, the Directorate also implemented the plan in two villages of Mandya district of Karnataka.

The activities of TSP under animal husbandry sector were related to distribution of small ruminants to tribal people, supply of mineral mixtures, feed supplements and arrangements of health camps in the villages wherein the tribes are having livestock population. A total of 280 sheep (240 in Karnataka and 40 in J&K), 12 bucks in Rajasthan, 124 piglets (70 in Meghalaya and 54 in Asom), 4,800 layer chicks in Kerala were distributed to the beneficiaries. Fifteen small poultry units were established in Madhya Pradesh for rearing of broiler chicks. Overall budget utilization and the report of the activities in different states were satisfactory.

TSP was initiated and implemented through this Directorate for the first time and continuous monitoring of the project is a challenging task. Creation of Sub TSP cells at each institute of ICAR and provision of man power assistance would bring in better results and the plan can be extended to some more areas of the country in relation to animal husbandry sector.

Dr S Ayyappan, Secretary DARE and DG ICAR visited PD_ ADMAS, Bangalore on 1st December 2011 and discussed various activities of the Directorate and advised to adapt latest technologies in Animal Disease Monitoring and Surveillance.

First Meeting of 3rd QRT

IMC Meeting

QRT Members visiting the laboratory

Dr. K.M.L. Pathak, DDG (AS) having discussion with the scientists

Dr. K.M.L. Pathak, DDG (AS) interacting with the scientists regarding the progress of the Projects

Release of 1st issue of PD_ADMAS News Letter by Dr. K.M.L. Pathak, DDG (AS), ICAR at XIX Annual Review meet AICRP on ADMAS at Jaipur, Rajasthan

Prize distribution in Hindi Week Celebration

Meeting of Consortium Implementation Committee of NAIP

Meeting of Consortium Advisory Committee of NAIP

Participation of TSP beneficiaries in the enterpreneurship development training programme under TSP

Implementation of TSP Project at Soligaradoddi Village with village participants

Participants of Model Training Course on Brucellosis

Inauguration of Training Programme on Animal Disease Informatics

Visit of Dr. H. Rahaman, Project Director to CSIRO, AAHL, Geelong, Australia

Participants of Training Programme on Animal Disease Informatics with dignitaries and Instructors

FAO Sponsored training programme on Spatial Epidemiology

Hindi Implementation Committee Meeting

Institute's Field Activities

CSF Investigation at Kirugavalu, Mandya, Karnataka

Visit of Scientist to Pig farm at Halebudnur, Mandya, Karnataka for CSF investigation

PD_ADMAS team in the flood affected areas of Odissa

Recording of Co-ordinates in Pig farm located at Induvalu, Mandya, Karnataka

Celebration of Institute foundation day

Project Director interacting with Gram Panchayat people at Shidlaghatta, Karnataka

ANNUAL REPORT
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Project Directorate
on
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Dr. H. Rahman, Project Director

Editors :

Dr. Divakar Hemadri
Dr. S.S. Patil

Front Cover :

Dr. M. R. Gajendragad

Decadal trend of Anthrax occurrence in India (1991-2010)

Back Cover :

Proposed new building of PD_ADMAS at Yelahanka, Bengaluru

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