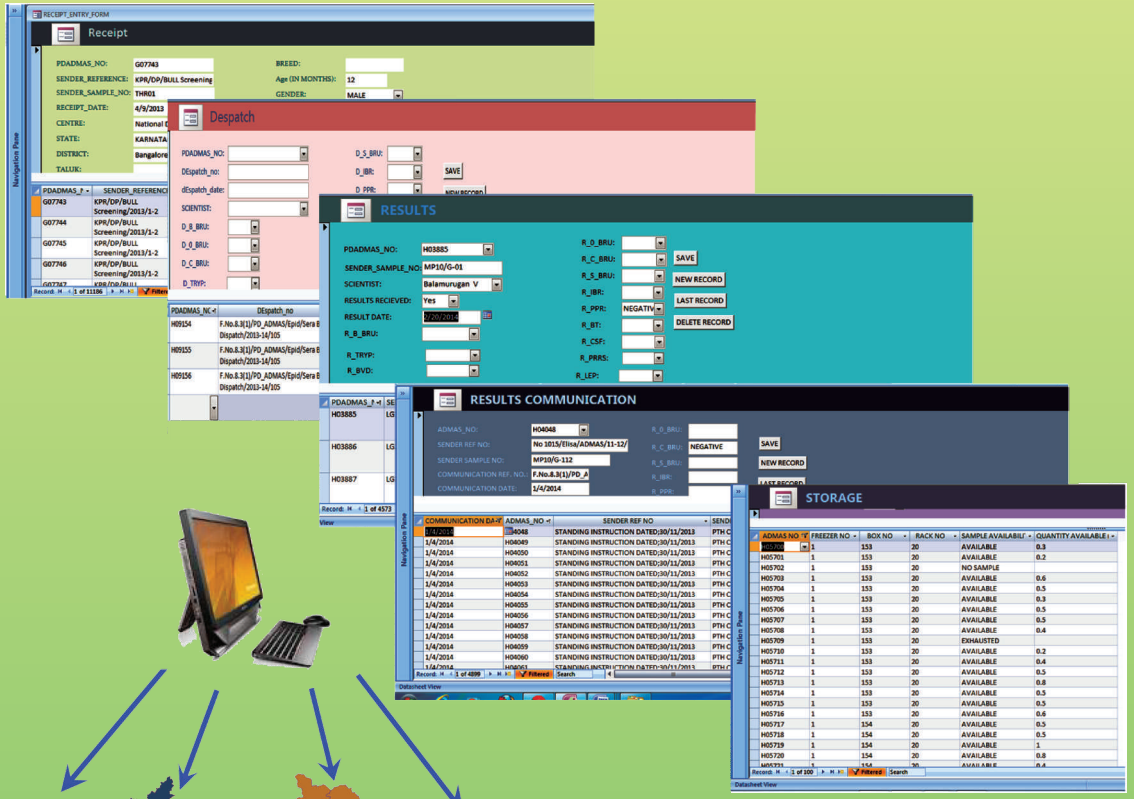
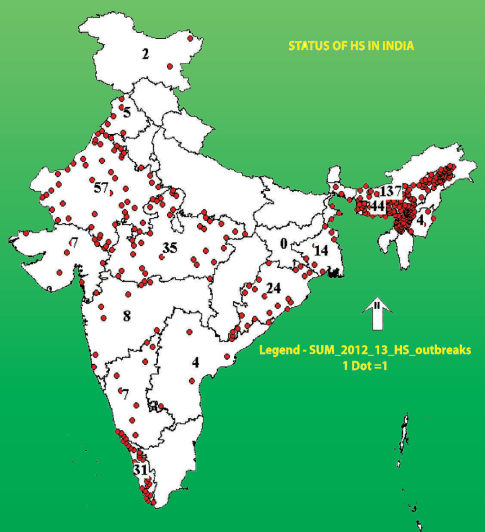
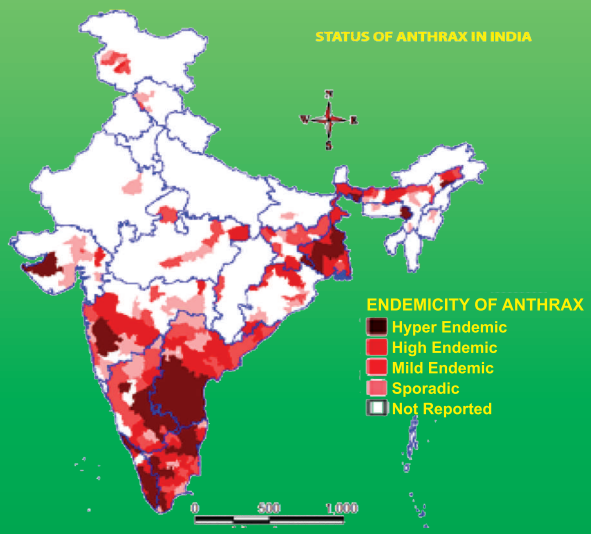
ANNUAL REPORT
2013-14

National Institute of Veterinary Epidemiology and Disease Informatics







National Institute of Veterinary Epidemiology and Disease Informatics (Formarly PD_ADMAS)
Hebbal, Bengaluru – 560 024, Karnataka, INDIA

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Cover Front Page : Generation of disease information
Cover Back Page : Genesis of NIVEDI

Printed at:
Sharadh Enterprises
Halasuru, Bengaluru - 560 003.
Phone : 080-25556015, 9845944311

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Acknowledgements

At the outset, the Director and all the staff members of National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI) express their sincere thanks and gratefulness to Dr. S. Ayappan, Secretary, DARE & Director General, ICAR for this constant support and lofty inspirations to this institute.

We also thank Dr. K.M.L. Pathak, Deputy Director General (Animal Science) and Dr. Gaya Prasad, Assistant Director General (Animal Health) for their continuous cooperation and encouragement and the support received from Dr. (Mrs) Jyoti Misri is also thankfully acknowledged.

Our sincere thanks are also due to the Director, NIANP; Director, NBAII; Joint Director, IVRI, Bengaluru; Head, Regional station, NBSSLUP, Bengaluru and Karnataka Veterinary Council and also other institutes and organizations for their vital logistic support and cooperation from time to time.

The Institute expresses sincere thanks to all the Principal investigators of AICRP on ADMAS and related state Animal Husbandry departments and universities for their excellent cooperation and inputs.

Last but not the least I sincerely thank all the staff members of NIVEDI for their support and cooperation.

Jai Jawan! Jai Kisan! Jai Vigyan!

(H. Rahman)
Director

Executive Summary

In India, the income from livestock sector is around ₹ 2075 billion which comprises about 4% of GDP of the entire country and 26% in agricultural sector. But due to economic losses derived from animal diseases, the entire growth in animal production may be jeopardized, slapping a big blow on the economic status of the nation as well as poor and landless farmers, which appear a significant loss of amount every year. Hence, constant effective animal health surveillance is pertinent to maintain a steady growth in this sector. As National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI) has been entrusted to study the epidemiology and disease surveillance in the entire country, its role is extremely pivotal for developing models of animal disease forewarning, forecasting, economic losses, risk analysis, precise diagnostics for better understanding of epidemiology, sustainable package of practices etc.

During the reported period NIVEDI has done extensive study on economically important livestock diseases viz. Peste des Petits Ruminant (PPR), Brucellosis, Infectious Bovine Rhinitis (IBR), Bluetongue (BT), Trypanosomiosis, Fascioliosis, Sheep and Goat Pox, Haemorrhagic Septicemia (HS), Rabies, Mastitis, Avian Influenza, Foot and Mouth Disease (FMD), Classical Swine Fever (CSF), Porcine Reproductive and Respiratory Syndrome (PRRS), Malignant Catarrhal Fever (MCF) etc.

NIVEDI is having an in-built interactive, dynamic web based software NADRES (National Animal Disease Referral Expert System). It is having both animal health information system (AHIS) and weather based animal disease forecast (WB_ADF). Thus the software provides both disease forecasting services and animal health information to the entire country especially those who are engaged in animal health sector.

This year around 5214 outbreaks data were uploaded to the website. During 1987-2014, retrospective analysis showed that FMD and sheep and goat pox remained top viral diseases, HS and Black Quarter (BQ) remained top bacterial diseases and fascioliosis and babesiosis were top parasitic diseases. Methodology was developed for assessing the risk of introduction of Notifiable Avian Inflenza (NAI: HPNA1 & LPNA1) in India. In a retrospective epidemiological study of HPAI during 2006-12, it was found that the majority of outbreaks was reported in West Bengal (55) followed by Assam (18). In a cross sectional surveillance study of MCF, 24.44 % sample were found positive for MCF infection among sheep (356) in Karnataka where Raichur district showed highest positivity. Among 6327 bovine serum samples, 52 % were shown positive for antibodies against IBRV in ELISA. In a retrospective study (1995-2014), Chhattisgarh showed highest seroprevalence (66%) with overall 36.52% seroprevalence in the country.

Poison normal model forecasting of PPR incidence in the states of Andhra Pradesh, Karnataka, Kerala and Maharashtra with the help of SAS NL MIX procedure was done. Besides, the methodology for deterministic model as enhanced solution for effective vaccination was developed by using basic reproductive number (R_0) for understanding the disease diagnosis. In retrospective study, it was revealed that 649 outbreaks were reported during 2003 - 13 in Karnataka. Highest outbreaks were reported in 2004 - 05. The economic loss analysis depicted that an estimated annual loss was ₹ 818.65 crores in India where as the same in Karnataka state was ₹ 60.13 crores. An overall 11.63% prevalence was recorded among goats in NE region indicating the area is endemic for PPR. A PPR clinical score card for the assessing the clinical disease pattern was developed which will be very useful for field investigation of PPR outbreak among sheep and goats in vaccinated and unvaccinated area. As per the available data, 73 outbreaks of sheep and goat pox were reported during 2000-07 in Tamil Nadu including 2133 attacks and 795 deaths in sheep and goats. The number of death was directly proportional to disease outbreaks and number of attacks. The outbreaks were observed more during December

to May in those years. On analysis of disease data on BT during 2001-14, the highest incidence was recorded during 2005-06. Most of the outbreaks were occurred during September to December. Spatial distribution of BT outbreak in Andhra Pradesh was mapped and as many as 7 serotypes have been reported during 2002-2012. In a study, out of 68 samples from domestic and wild animals, 36 samples were recorded positive for rabies by dFAT and RT-PCR. On partial gene sequencing all the isolates were found belonged to classical rabies virus genotype1.

An overall 42.8 % pig sample (collected from Madhya Pradesh, Andhra Pradesh, Kerala, Assam, Manipur, Meghalaya and Karnataka) were found positive for the presence of antibodies against CSFV by ELISA. The overall seroprevalance of 35.4 % during 2010-14 was recorded. Besides, 29.9 % sample was recorded for the presence of antibodies against PRRS virus infection among 352 pig serum samples collected from Assam, Arunachal Pradesh, Meghalaya, Manipur, Nagaland, Maharashtra and Karnataka. In Karnataka, during 2013-14, FMD outbreaks were recorded in all districts except Gulbarga and Yadgir districts, a total of 5514 villages of 29 districts were affected; the highest number of bovine were affected in Kolar district (9817) and lowest in Bijapur district(43). The mean mortality rate was 15.57 %. During 2001-12, in Karnataka, HS was occurred alone in Hassan, Chitradurga and Davangere districts and concurrent with FMD in Nellore and Guntur districts in Andhra Pradesh. Secondary data on FMD was collected from different districts of India and mapped in high medium and low risk grades. It was observed that HS occurred mostly during July, August and September but FMD occurred throughout the year. The economic losses due to HS in Karnataka among cattle and buffaloes was estimated as ₹ 23.30 million during 2001-02 which decline to ₹ 1.97 million during 2012-13.

In DBT network project on brucellosis, real time PCR assay and multilocus sequence typing of *Brucella* was done. Among animals buffaloes showed highest positivity for brucellosis followed by HF cross bred. In another study, 7.38 %, 6.04 %, 7.04 % and 10.06 % were found positive by MRT, ELISA, RBPT and serum ELISA, respectively among 298 serum samples tested. A battery of recombinant antigens targeting BLS, bp26, sodc gene of *Brucella abortus* has been developed. Besides, 4.14 % serum samples were found positive among 9195 animals (cattle, buffalo, sheep, goat and pigs) in ELISA. A total of 64818 pooled milk samples collected from the Karnataka were subjected to MRT and 2.55 % positivity was observed. A protein G based ELISA kit for detection of brucellosis were evaluated and found 88.79 % sensitivity, 97.74% specificity. A lateral flow assay for detection of brucellosis was also developed and a good agreement was found when compared with RBPT and indirect ELISA. Among 295 animal and human serum samples tested highest seropositvity was found with *Leptospira* followed by *Brucella* and *Listeria*. A lateral flow assay for the detection of listeriosis has also been developed with high diagnostic efficacy among human population. In a non-risk human population, 9% seroprevalence of brucellosis was recorded and the age group of 31- 40 years and 51- 60 years were found more vulnerable to brucellosis. For human neurobrucellosis, the age group of 21 - 30 years and 31 - 40 years were noted more susceptible to this disease. A recombinant antigen LigB protein (*Leptospira borgpetersonii* Hardzo serovar) based ELISA was developed. This antigen showed 88.24% sensitivity and 95.4% specificity in ELISA when compared with MAT. Seroprevalence of leptospirosis were studied in different coastal regions of India including east, south and west and 24.96 % was found positive among 1309 samples. The highest prevalence recorded in WB (80.9%). In a study, 16 isolates of *E. coli* were found for MBL positive, among 9 isolates were also found positive for ESBL. Different gene detection of MBL in 10 isolates was done but none found positive and none of 9 ESBL *E. coli*, were found positive for *ampC* gene. Besides, *in vivo* antibacterial efficacy of new vancomycin derivative showed dose dependent activity against MRSA and VISA in mice. In another study, TLR2 and TLR 4 in Deoni and HF breed were analysed by PCR assay. The clusterW multiple sequence

alignment of complete exonic region of TLR 2 gene in both the breed reveals that out of 23 SNPs, 19 SNPs were unique to Deoni, 3 SNPs to HF and one SNP was shared among both the breeds.

In a serosurvey for the surra antibodies by ELISA, 51.06% sample for bovine was recorded as positive. The samples were collected from West Bengal, Karnataka, Tamil Nadu, Odisha and Madhya Pradesh. In camels, an overall 21.4% among 626 serum samples was found positive. The samples were collected from Jaipur, Udaipur, Jaisalmer, Ajmer, Barmer, Bikaner and Hanumangarh districts of Rajasthan. The evaluation of immunoreactivity of recombinant antigens (VSG and ISG proteins) of *Trypanosoma evansi* was carried out. The antigens showed high degree of sensitivity and specificity (more than 90%) in ELISA. The monoclonal antibodies against these proteins were also developed and they showed immuno reactivity both in ELISA and immunoblot assay. On isotyping analysis, the monoclonal antibody showed IgG2b and IgG3 type. Besides, among 304 feacal samples collected from cattle, buffaloes, sheep and goats from Karnataka, West Bengal and Odisha, 37% were found positive for *Fasciola* infection. In another study, 38% *Lymnea spp*. snails (collected from West Bengal and Karnataka) were found positive for the presence of *Fasciola* infection by PCR assay.

During 2013-14, 12548 serum samples comprising cattle (5498), buffaloes (1824), goats (3263), pigs (520), sheep (1443) were received, catalogued and arranged by serum bank. These serum samples were further screened for presence of antibodies against different pathogens and observed positive viz. *Brucella abortus* - 3.44%; *Brucella suis* - 7.9%; *Brucella melitensis* - 11.73% in sheep and 4.46 % in goats; BoHV1 - 47.59%; CSFV - 41.89 %.

About NIVEDI

The project on animal disease monitoring and surveillance (ADMAS), P1 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 the 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 X plan. In XI plan Guwahati Centre in Assam has been included as a collaborating unit of AICRP on ADMAS. Keeping in view of the tremendous contributions of this directorate to country's livestock health sector, the council upgraded it to a National Institute and rechristened as "National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI)" on 25th October 2013 in the XII plan.

Research mandates of NIVEDI

* 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

* Seromonitoring 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 Repository.
* 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.

Institute Research Projects

IPC: ANSCNIVEDISIL201200900033 Project ID: IXX09487

Modeling and Forecasting the Incidence, Prevalence and Outbreak of PPR in India

K. P. Suresh, V. Balamurugan, S. S. Patil, D.Hemadri and G.Govindaraj

Developing accurate, real-time, geographic, early Incidence or outbreak-forecasting systems are imperative in the present condition for epidemic preparedness and prevention of PPR. Stochastic and deterministic models were fitted to PPR data derived from NADRES, an official database of NIVEDI. The count models such as Poisson Normal models were examined for the data. Letting denote the number of failures for the th pump in the th group, Draper (1996) considers the following hierarchical model for these data:

$y_{ij}|\lambda_{ij} \sim$ Poisson (λ_{ij})

$\log \lambda_{ij} = a_i + \beta_i (\log t_{ij} - \log t) + e_{ij}$

$e_{ij}|\sigma^2 \sim$ Normal $(\theta_1 \sigma^2)$

The model specifies different intercepts and slopes for each group, and the random effect is a mechanism for accounting for over dispersion by using SAS. Results from the analysis revealed that the poison normal models fitted very well with low SE as depicted in the table 1.

Table 1 : Results of poison models for the four states studied

	Andhra Pradesh		Karnataka		Kerala		Maharashtra	
	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
Logsig	1.29	0.06	1.61	0.11	2.04	-	1.98	0.12
Beta	0.04	0.66	8.83	1.43	-2.55	0.42	1.01	1.15
Alpha	2.75	2.70	-38.49	6.27	-2.89	3.04	-7.86	4.66

Deterministic models as enhanced solution for effective vaccination.

The following methodology was developed for computing basic reproductive Number (R_0). R_0 is affected by several factors like the duration of infectivity, infectiousness of the organism, number of susceptible animals with whom the infected animal comes in contact etc., In general, if R_0 is greater than one, the disease will continue to spread within a population. If R_0 is less than one, the disease will eventually disappear from a population. R_0 is calculated using the formula $R_0 = C * P * D$, where

C = the number of contacts the infectious animals makes per unit time (day, week, month, etc.)

P = the probability of transmission per contact with the infectious animal

D = the duration that the infected animal is infectious to others or average duration of infection (ADI).

The Average duration of infection is further estimated using the following epidemiological parameters viz., maximum and minimum duration of incubation period (IP), maximum and minimum duration of disease course (DC) over all forms, maximum and minimum case fatality rate (CFR), proportion of surviving animals which become latently infected (LIS), maximum duration of latent infection (latent period: LP).

Uniform distribution is used to represent each of these variables

Mean = (b+a)/2 and Variance= (b-a)2/12

ADI=(IP+DC)X(CFR)+(IP+DC)*(1-CFR)x(1-LIS)+(LP)x(LIS)

Using this model the Ring or Disease cluster will be identified by calculating the R_0 with a specified radius of cluster.

IPC: ANSCNIVEDISIL201200200026 Project ID: IXX07978

Economic Analysis of Haemorrhagic Septicemia (HS) in Cattle and Buffaloes in Selected Endemic States of India

G. Govindaraj, M. R. Gajendragad and P. Krishnamoorthy

During the year 2013-14 collection and analysis of primary data from Karnataka state was undertaken. Multi stage random sampling technique was adopted for the primary survey to assess loss due to HS disease in cattle and buffaloes. The results revealed that, majority of the animals were owned by OBC category farmers compared to other group of farmers. In majority of the farms the disease persisted for less than three days, while in some farms it persisted for 4-6 days and in few farms it persisted for more than 7 days (Fig. 1). The results of month wise occurrence revealed that among the sample households the disease occurred round the year except in March. Though the disease occurs round the year, in majority of the farms infection was during monsoon period (May-August) and also during the winter months (November-January) (Fig. 2). We can infer from the above result that in the study area HS disease is predominant in rainy and winter months. Majority of the infected farms, feeding habit was poor to moderate type and it might have caused some stress to the animals. Majority of the households in the study area do not provide any mixture supplements causing nutritional stress. Mortality and morbidity loss due to HS in Karnataka state revealed that the estimated loss due to HS was ₹ 23.30 million during 2001-02 and it declined to ₹ 1.97 million during the year 2012-13.

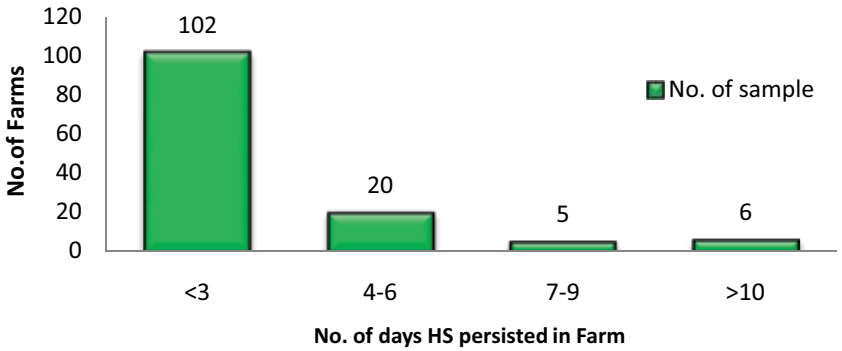

Fig 1. Farm level persistency of HS disease

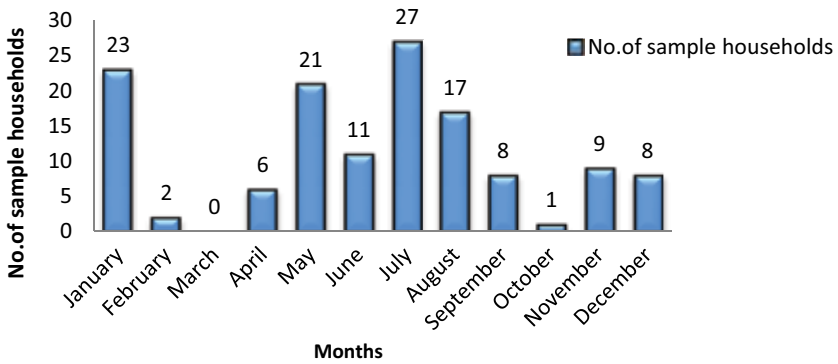

Fig 2. Occurance of HS in different months

IPC: ANSCNIVEDISIL 201201000034 Project ID: IXX09422

Epidemiology of Haemorrahgic Septicemia in Livestock vis-à-vis Foot and Mouth Disease in India

P. Krishnamoorthy, B.R. Shome and G. Govindaraj

Secondary data on Haemorrhagic septicemia (HS) and Foot and Mouth Disease (FMD) outbreaks were collected from Madhya Pradesh, Andhra Pradesh, Karnataka and Tamilnadu, and morbidity and mortality rates were calculated. The year wise HS and FMD outbreaks occurred during 2005 to 2012 in Madhya Pradesh showed a constant trend of disease and based on month-wise occurrence, HS occurred mostly during July, August and September months and FMD occurred throughout the year. Madhya Pradesh showed morbidity rate of 14.68 per 10^4 populations for HS during 2005-12.

In Madhya Pradesh, only HS outbreaks have occurred alone in many districts during the year 2005-12. In Andhra Pradesh, continuous occurrence of HS and FMD outbreaks in Nellore and Guntur during 2002-12 was observed. In Tamilnadu, increased occurrence of HS outbreaks in Kancheepuram and Tiruvannamalai and FMD outbreaks in Madurai, Trichy and Tiruvannamalai was observed. In Karnataka, Hassan, Chitradurga and Davanagere districts showed increased number of HS outbreaks. The districts showing continuous outbreaks should be concentrated for vaccination so that the outbreaks can be reduced or prevented. HS outbreaks occurred mostly in districts which do not have FMD outbreaks were observed in various states. On analysis of district wise occurrence of HS and FMD, some districts showed occurrence of HS outbreaks alone and not the FMD which suggests there may not be co-occurrence of the diseases. The number of HS and FMD outbreaks occurred during 2012-13 in different states of India was mapped (Fig. 3). The map showed that the HS and FMD outbreaks occurred in different numbers in different states.

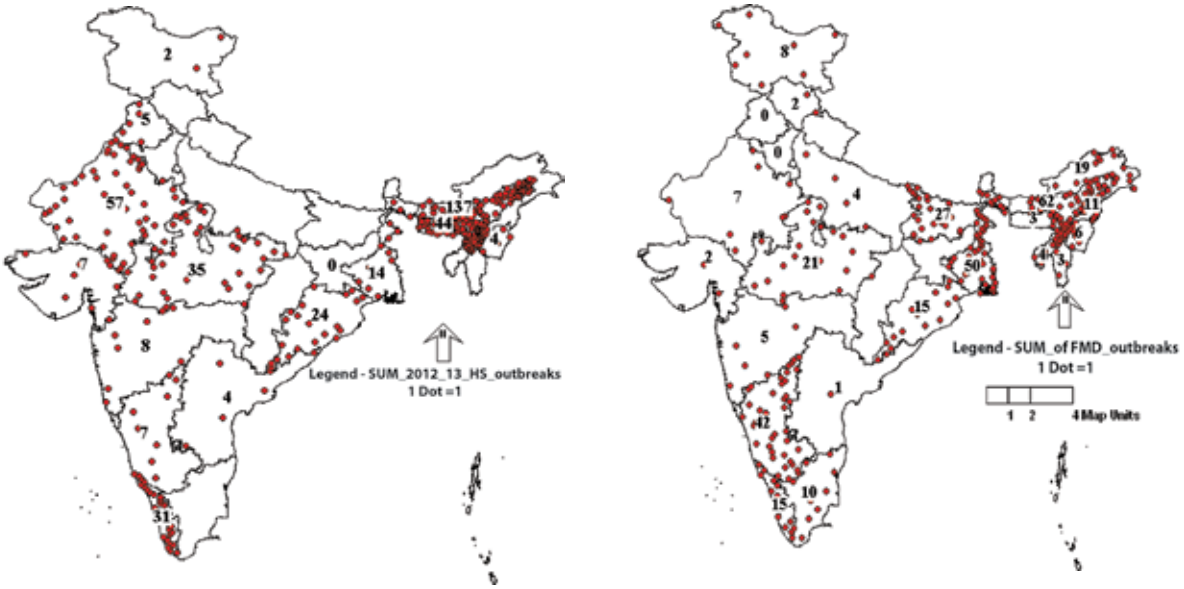

Fig. 3

The Foot and mouth disease outbreaks during 2013-14 occurred in all the districts in Karnataka state except Gulburga and Yadgir districts. The total number of villages affected was 5514 in 29 districts

of Karnataka state. The number of animals affected by the disease is highest in Kolar (9817) and lowest in Bijapur district (43). The mean mortality rate due to the FMD in Karnataka during 2013-14 is 15.57 %. 44 blood and 19 swabs samples collected from FMD suspected and ailing animals and tissue samples from three dead animals from Bangalore rural, Chamrajanagar, Hassan, Mandya and Ramanagaram in Karnataka. The samples were found negative for *Pasteurella multocida* by isolation, and species and type B specific multiplex PCR. Twenty eight sera samples from bovines suspected for FMD from Mandya and Ramanagaram districts of Karnataka were collected. The samples were tested for FMD antibodies by DIVA ELISA by IAH&VB, Bangalore. Sixteen samples (57.14%) were found positive for FMD, in which three cattle had history of FMD vaccination. This indicated that there is no occurrence HS in FMD affected cattle in karnataka alone in these outbreaks in Karnataka. This further emphasised that there is no co-occurrence of FMD and HS outbreaks.

IPC: ANSCNIVEDISIL201000200019 Project ID: IXX01081

Epidemiology of Bovine Leptospirosis by Using Recombinant Antigen Based Assays

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

This project has been designed with the objective of expression of LigB protein and to evaluate its suitability as antigen for diagnosis of bovine leptospirosis in diagnostic tests such as enzyme linked immuno sorbent assay (ELISA) and latex agglutination test (LAT). The truncated region of (883-1935 bp) *ligB* gene of *Leptospira borgpetersonii* Hardjo serovar was amplified by PCR from DNA sub-cloned into pET32a vector and introduced into BL21 *E. coli* cells for expression. The expressed protein was purified using Cobalt affinity column. This antigen is used in ELISA and LAT which were further standardized. OMP proteins and heat killed leptospiral antigen were also produced which were further used in LAT for comparison.

A total of 390 cattle serum samples (85 positive and 305 negative by MAT) have been used for standardizing rLigB based ELISA and 214 samples (108 positive and 106 negative by MAT) have been used for standardizing LAT.A 2 X 2 contingency table was presented for LAT using all the three proteins separately against MAT for 214 serum samples. In addition performance of LAT in serum various species which had given encouraging results (Fig. 4).

In ELISA, based upon test samples, ROC curve was drawn using Winepiscope 2.0 software. Based upon ROC curve analysis for rLigB based ELISA, area under curve is 98.010 (97.460-98.560) and a cut off at PP value 30, sensitivity is 88.24 % and specificity is 95.41 % (Fig. 5). Similarly, a total of 214 samples have been subjected for MAT, rLigB based LAT, OMP based LAT and heat killed leptospiral protein based LAT. Evaluations of all the tests are carried out through software Winepiscope 2.0.

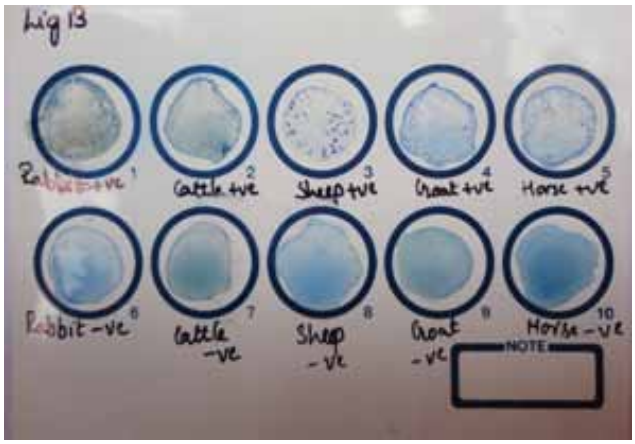

Fig. 4: Latex agglutination test using rLigB antigen in rabbit, cattle, sheep, goat and horse

Comparing sensitivity and specificity of different tests, rLigB ELISA is having higher sensitivity and specificity in relation with MAT and hence can be easily used in laboratories which have equipments

and expertise for ELISA. Based on Kappa value analysis, moderate agreement exists between recombinant LigB based LAT, OMP based LAT and heat killed leptospiral protein based LAT with MAT. In spite of moderate agreement, it can be used at field level where there is no facility for MAT and other diagnostic facilities available for screening of leptospirosis.

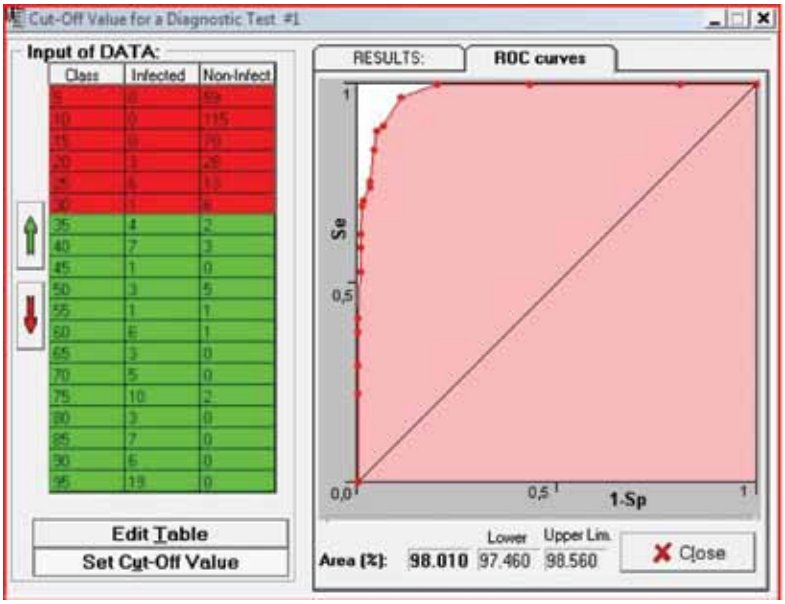

Fig. 5: Area under curve for LigB based ELISA against MAT by ROC Curve analysis using Winepiscope 2.0 software.

IPC: ANSCNIVEDISIL201100200021 Project ID: IXX08329

Molecular Epidemiology of MRSA, MR-CoNS and ESBL producing Gram-negative Bacteria in Animals including their Environment

B. R. Shome and R. Shome

A total of 9 *E. coli* isolates that showed resistance / intermediate resistance against one of the cephalosporins (cefotaxime, ceftazidime, ceftriaxone) or monobactams (aztreonam) were considered to be ESBL positives. Among these 9 ESBL producers 6 isolates also showed resistance/intermediate resistance to imipenem (IPM) and hence were considered as MBL producers. A total of 10 other *E. coli* isolates showed to be MBL producers. To investigate the genes encoding these enzymes in the phenotypically identified ESBL and MBL producing strains of *E. coli*, PCR based molecular screening was carried out as follows:

Molecular Detection of ESBL genes: PCR based molecular screening was carried out to dectect the β-lactamase genes viz., TEM, SHV, CTX-M-I, II, III, IV groups using published primers (Pitout *et* al., 2004, Kojima, 2005) in 9 ESBL *E. coli* isolates. All 9 ESBL *E. coli* isolates were screened for ampC genes also using the published primers (Perez et al, 2002).

For detection of TEM and SHV gene, a uniplex PCR was performed. Only one isolate (pig fecal origin) was positive for SHV gene and none of the 9 ESBL isolates were positive for TEM (Table 2).

Similarly for detection of CTX-M-I, II, III, IV groups, uniplex PCR was performed as above. One isolate (pig fecal origin) was positive for both CTX-M I and CTX-M III group. None of the isolates were positive for CTX-M II and CTX-M IV groups.

Table.2: PCR screening of ESBL genes

Sl No.	Isolate ID	TEM	SHV	CTXM-1	CTXM-II	CTXM-III	CTXM-IV
1	N1	-	-	-	-	-	-
2	F4	-	-	+	-	+	-
3	F8	-	-	-	-	-	-
4	F14a	-	-	-	-	-	-
5	F16	-	-	-	-	-	-
6	F17b	-	-	-	-	-	-
7	F19b	-	-	-	-	-	-
8	F22	-	-	-	-	-	-
9	F23b	-	+	-	-	-	-

For detection of ampC genes, uniplex PCR was performed as per protocol above except 0.6μM of each primer (MOXF-MOXR, CMYF-CMYR, DHAF-DHAR) and 0.5μM of each primer (ACCF-ACCR, EBCF-EBCR and FOXF-FOXR). None of the 9 ESBL *E. coli* isolates were positive for ampC genes.

Molecular Detection of MBL genes: PCR based molecular screening was carried out to dectect the metallo-β-lactamase genes viz., IMP, VIM, GIM, SIM and SPM using published primers (Mendes et al., 2007) in 10 MBL *E. coli* isolates. None of the 10 *E. coli* isolates were found positive for MBL genes.

Determination of in vivo antibacterial activity of new vancomycin derivatives

A separate single-dose study of new vancomycin derivatives (JNCASR, Bengaluru) was performed in neutropenic mice infected in the thigh with MRSA or VISA (10^7 CFU/ml). Infected animals were treated intravenously, at 1 hr post infection, with 2, 4, 8 and 12 mg/kg. At 24hr post infection mice were sacrificed and the thigh tissues were harvested for the bacterial titer.

The in vivo efficacy of new vancomycin derivatives I and II were evaluated in a neutropenic mouse thigh infection model. In this study, mice were infected with VISA (10^7 CFU/ml) in the thigh. After 1 hr of infection the mice were treated with intravenously administered vancomycin, linezolid and new vancomycin derivatives at 12 mg/kg, saline being used as control. After 24 hr of the initial treatment, antibacterial activity was determined by finding the bacterial titer in the infected thighs. New vancomycin derivatives showed excellent activity against VISA, whereas vancomycin and linezolid were completely ineffective at 12 mg/kg (Table 3).

Table 3: In vivo antibacterial activity of new vancomycin derivatives against VISA in mice neutropenic thigh infection model

Bacterial species	Drug administered (i.v.)				
	Saline	Linezolid (12mg/kg)	Vancomycin (12mg/kg)	New derivative I (12mg/kg)	New derivative II (12mg/kg)
VISA	$1.36x10^9$	$3.65x10^8$	$1.14x10^9$	$4.7x10^5$	$4.5x10^4$

In another study, the effect of dose response on the efficacy of new vancomycin derivative I and II were performed in the mouse neutropenic thigh MRSA or VISA infection model. After 1 h of infection, a single dose of new vancomycin derivatives at different regimens (2, 4, 8 and 12 mg/kg, i.v.) was administered. The pretreatment bacterial titer in the thigh was 7.2 ± 0.2 log10 CFU/g. In vehicle treated controls, thigh titer increased to 9.1 ± 0.1 log10 CFU/g within 24 hr. New vancomycin derivatives produced comparable dose dependent reductions in the bacterial titer at each of four dosing regimens that are summarized in Table 4 and 5.

Table 4: Dose-dependent in vivo antibacterial activity of new vancomycin derivative I against MRSA in mice neutropenic thigh infection model

Bacterial species	Bacterial Count (CFU/g)				
	Saline	2mg/kg	4mg/kg	8mg/kg	12mg/kg
MRSA	$1.672x10^9$	$6.26x10^7$	$1.2x10^6$	$4.83x10^4$	$14x10^4$
VISA	$1.29x10^9$	$2.02x10^8$	$1.82x10^7$	$5.4x10^6$	$4.8x10^4$

Table 5: Dose dependent in vivo antibacterial activity of new vancomycin derivative II against VISA in mouse neutropenic thigh infection model

Bacterial species	Bacterial Count (CFU/g)			
	Saline	2mg/kg	4mg/kg	12mg/kg
VISA	$3x10^{10}$	$2.5x10^7$	$1.1x10^6$	$4.8x10^4$

The present study revealed that new vancomycin derivatives have dose-dependent *in vivo* efficacy against MRSA and VISA.

IPC: ANSCNIVEDISIL201200100025 Project ID: IXX08032

Epidemiological Survey and Estimation of Economic Impact of PPR in Sheep and Goats

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

Epidemiological analysis of the PPR outbreaks in Karnataka

Time series data from 2003 to 2013 years PPR outbreaks data from Karnataka was collected and analyzed the epidemiology of the PPR and also estimated economic losses. In Karnataka, the majority of the population lives in rural areas and depends on animal husbandry for their livelihood. Sheep and goats are kept largely by low-income, landless villagers for whom such activity provides the main source of income. The disease was reported in the state for the first time in 1992 and is considered to be the major threat to the small ruminant population and hence to the farmers. Between 2003 and 2013, a total of 649 PPR outbreaks were reported from different districts of Karnataka. The highest number of outbreaks was reported between 2004-05 and the second highest number was reported in 2005-06 and between 2011-12, less outbreaks were reported. The reduction in the number of outbreaks in 2006 and 2007 suggests that the large-scale preventive vaccination carried out in the state for control of disease. Analysis of the monthly occurrence of the disease showed that till August, there was few outbreaks but there after number increased slowly. Some researcher opined that onset of the monsoon may be a factor for increase in outbreaks. Rainfall causes animals to huddle together, enhancing close contact (a major transmission route for PPR), and high relative humidity values are associated with virus survival in aerosols. Movement of animals due to increased sheep trade also increases the disease incidence. The sheep and goats population status and the progression of PPR outbreaks since 2003 is shown in Fig. 6.

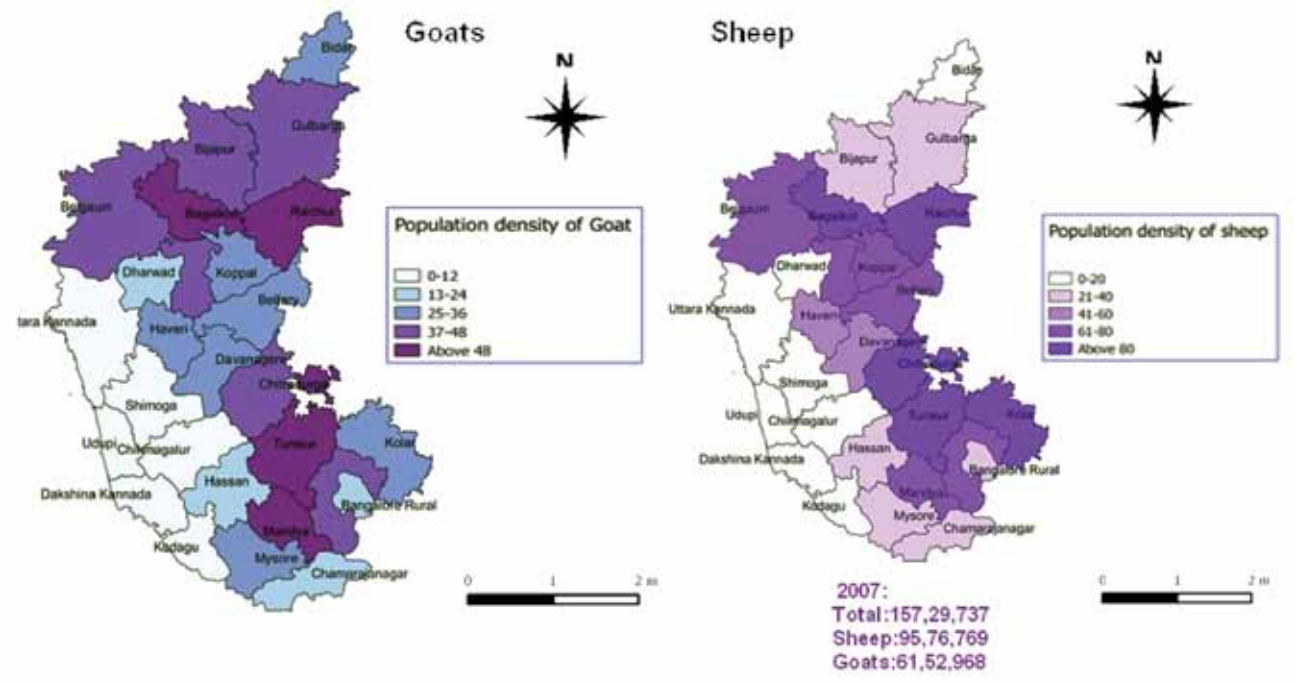

Fig.6 Goat and Sheep population in different districts of Karnataka

Source : Office of the Deputy Director, Rinderpest Eradication Scheme (RPES), Deparment of Animal Husbandry and Veterinary Services, Bengaluru

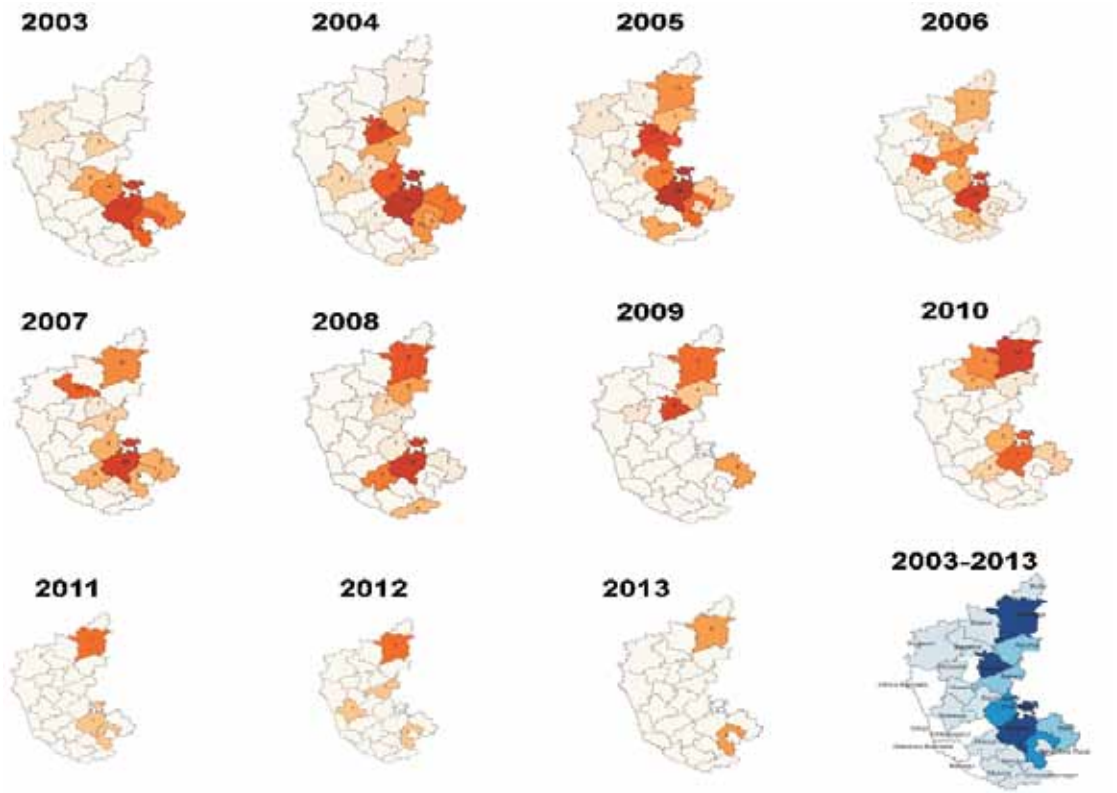

Fig. 7

PPR clinical score card for assessing the clinical disease pattern

PPR clinical score card was developed based on the certain scientific inputs acquired during field investigation of outbreaks and assumptions for assessing disease severity pattern during PPR outbreaks in field conditions. Analyses of primary data collected during outbreaks were used for the evaluating the developed clinical scorecard. This card will be useful in assessing the severity of the disease pattern like severe, moderate, mild etc., during PPR outbreaks in sheep and goats in the vaccinated and unvaccinated area.

Estimation of economic loss due to PPR in sheep and goats

The direct and indirect economic loss due to PPR in sheep and goats was estimated using the mathematical models. The parameters considered for assessing the loss due to PPR were adult and young populations, PPR prevalence, mortality and morbidity percentage etc. The estimated annual economic loss due to PPR in sheep and goats was ₹ 818.65 crores in India and ₹ 60.13 crores in Karnataka state. The spread sheet model using excel was also developed for estimation of economic loss due to PPR infection in sheep and goats in India and other states. Estimation of outbreaks losses due to reported PPR infection in sheep and goats in Karnataka and Andhra Pradesh state were also carried out. Further, secondary data was collected on the national control programme of PPR from Karnataka state for assessing the impact of the ongoing NCP-PPR programme in Karnataka and Andhra Pradesh. Since this estimation was based on the a nominal 5% incremental PPR prevalence, it may considered as best estimate until the estimate based on appropriate sampling methods and designs to arrive, to generate authentic data or unless the perfect reporting animal disease ensured at different field level. The approaches used in estimating the loss due to PPR in sheep and goats in Karnataka state with present study will be extended for other study to estimate the losses due to PPR. Appropriate spread sheet modules will also be developed for different states for estimation of losses due to PPR.

IPC: ANSCNIVEDISIL201201800042 Project ID: IXX09665

Epidemiology and Impact Analysis of Sheep and Goat Pox

G. B. Manjunatha Reddy, S. S. Patil, V. Balamuragan and D. Hemadri

Secondary data on sheep and goat pox disease was collected from the animal husbandry directorate of Tamil Nadu. The data was restructured according to number of disease outbreaks, number of attacks, number of deaths, month and year wise. The graphs are constructed using suitable software and data was interpreted. Pox outbreaks were attended and samples and data were collected from six different districts in Karnataka. The disease was diagnosed by post-mortem examination, histopathology and PCR. The virus was confirmed by sequencing and phylogenetic analysis.

The data collected (Tamil Nadu) included number of outbreaks, number of attacks and number of deaths at village level. The disease data collected was stratified in to block, district, region, month and year-wise. A total 73 pox outbreaks were reported from 2000-2007 from different districts with 2133 attacks leading to 795 deaths in sheep and goat in Tamil Nadu. There was increasing trend in number of disease outbreaks from 2000 to 2005 then was decline in trend was observed (Fig. 8). The number of deaths was directly proportional to disease outbreaks and number of attacks. The sheep and goat pox disease outbreaks were more recorded during December to May months (Fig. 9). The disease was confirmed by PCR targeting P32 gene. The sequencing and phylogenetic analysis showed

close relation with published Indian sheep and goat pox virus isolates. In conclusions the sheep and goat pox disease outbreaks were recorded mostly during summer with mortality rate of 37.27%. The disease outbreaks were recorded in unvaccinated flocks especially after transport and new stock introduction. The P32 gene based PCR for diagnosis of pox disease was standardized.

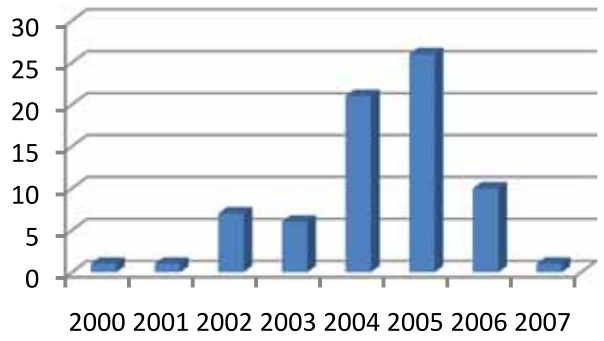

Fig. 8

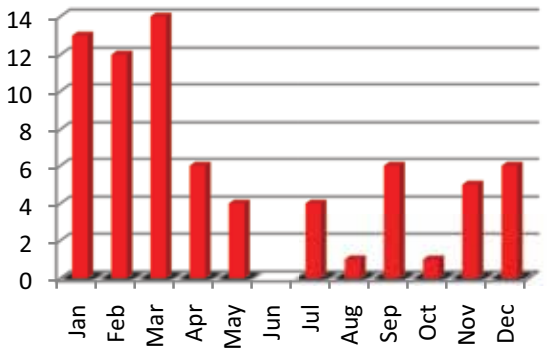

Fig. 9

IPC: ANSCNIVEDISIL201100400023 Project ID: IXX07919

Epidemiology of Classical Swine Fever in India

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

A total of 373 pig serum samples from 7 states viz., Kerala (36), Manipur (87), Meghalaya (51), Karnataka (43), Assam (52), Andhra Pradesh (46), and Madhya Pradesh (58) were tested for the presence of antibodies against classical swine fever infection using ELISA kits during the period under report. 160 serum samples were found positive for CSF antibodies (42.8%) (Table 6). All the serum samples were from the unvaccinated pigs. Screening of 3166 pig serum samples from 2010-14 revealed a CSF prevalence of 35.4% (Table 7) in the country. Analysis of weather parameters in some parts of southern Karnataka revealed the presence of ambient temperature (27-28^{0}C) favoured the long persistence and transmission of CSF virus in the region. The procurement and supply of piglets was from the farms having no proper health records (like vaccination status). It was also understood that the piglets were procured from neighbouring states viz,. Kerala, Andhra Pradesh and Tamil Nadu.

Phylogenetic analysis of 5'UTR, NS5B and E2 genomic regions of PCR amplicons obtained from pig tissues belonging to Karnataka, Andhra Pradesh, Maharashtra, Punjab, Odisha and Arunachal Pradesh revealed the emergence of CSFV subtype 2.2 (Fig. 10) The cumulative seroprevalence of CSF from 2010-14 was 35.4% in India. Phylogenetic analysis of 5'UTR, NS5B and E2 genomic regions of Indian isolates revealed the emergence of CSFV subtype 2.2.

Table 6: State-wise seroprevalence of CSF in India during 2013-14

Sl. No.	State	No. Tested	No. Positive	No. Negative	% Percentage
1	Kerala	36	15	21	41.6
2	Manipur	87	25	62	28.7
3	Meghalaya	51	16	35	31.3
4	Karnataka	43	16	27	37.2

5	Assam	52	35	17	67.3
6	Andhra Pradesh	46	22	24	47.8
7	Madhya Pradesh	58	31	27	53.4
	Total	373	160	213	42.8

Table 7: Cumulative seroprevalence of CSF in India during 2010-14

Sl No	Year	No. Tested	No. Positive	No. Negative	% Positivity
1	2010-11	1257	237	1020	18.85
2	2011-12	426	191	235	44.83
3	2012-13	1110	535	575	48.19
4	2013-14	373	160	213	42.8

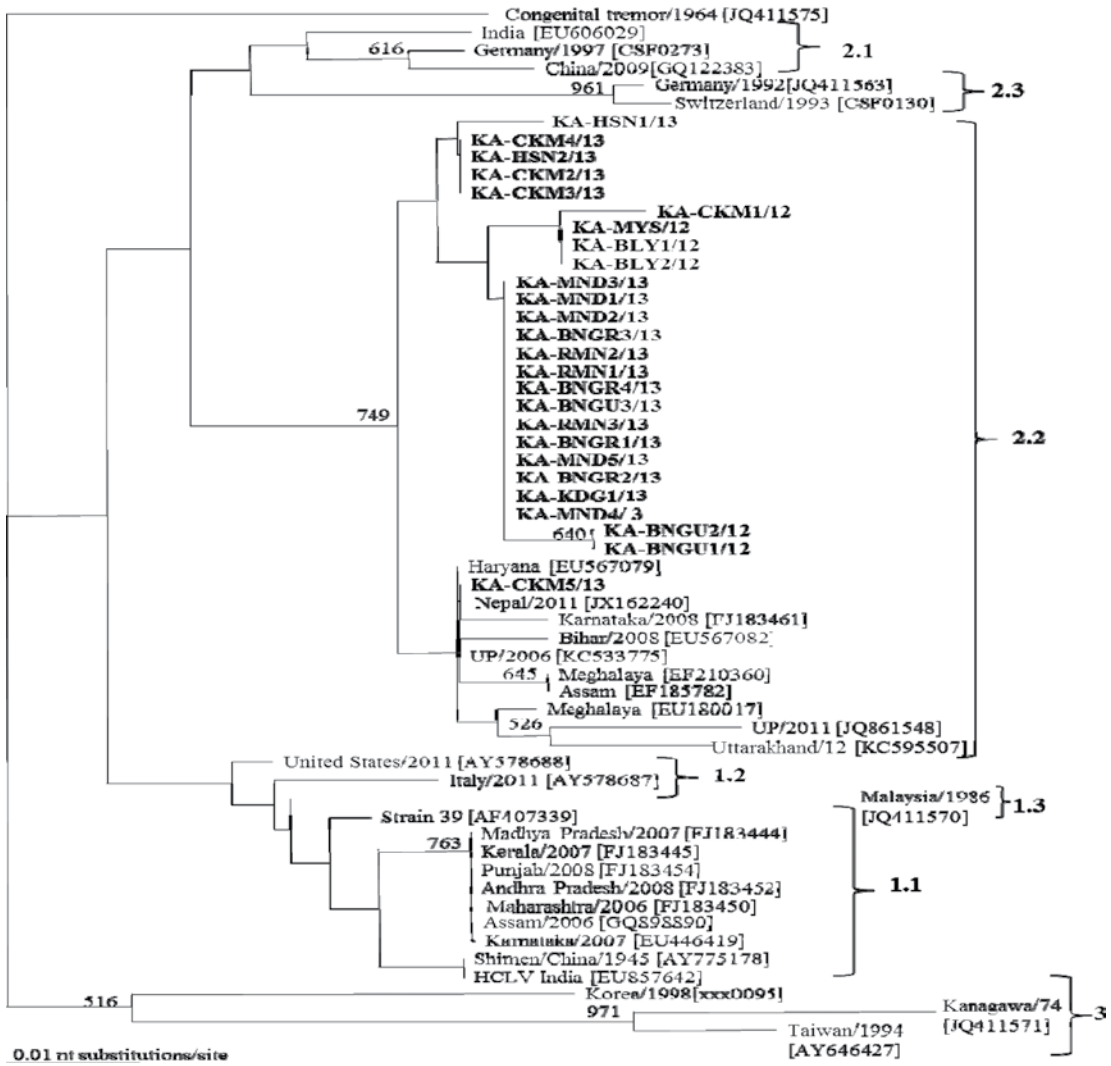

Fig. 10: Phylogenetic tree based on the analysis of 150 nucleotides within 5'UTR

Porcine Reproductive and Respiratory Syndrome (PRRS)

PRRS is highly contagious disease of pigs caused by PRRSV belonging to the genus Arterivirus, family Arteriviridae and order Nidovirales. This economically important, panzootic disease causes reproductive failure in breeding stock and respiratory tract illness in young pigs. A total of 652 pig serum samples from Assam, Arunachal Pradesh, Meghalaya, Manipur, Nagaland, Karnataka and Maharashtra were screened for the presence of antibodies against PRRSV infection using ELISA kit during 2012-14. 285 serum samples were found positive for PRRS antibodies (29.9%) (Table 8).

In conclusion, more number of pig serum samples to be screened on routine basis for establishing the source of PRRSV infection.

Table 8: The cumulative seroprevalence of PRRS in India during 2012-14

Sl No	Year	No. Tested	No. Positive	No. Negative	% Positivity
1	2012-13	851	285	566	33.49
2	2013-14	101	0	101	0
	Total	**952**	**285**	**667**	**29.9**

IPC: ANSCNIVEDISIL201100500024 Project ID: IXX07976

Epidemiological Study of Surra and Fasciolaisis in Animals

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

Surra and Fascioliosis are two important vector borne diseases of Indian livestock including bovine, equine, dromadaline, feline, etc. Surra is caused by *T. evansi*, transmitted by tabanid fly. Tropical facioliosis is caused by digenetic tramatode - *F. gigantica*, which is transmitted by *Lymnia spp.* Snail host, can affect wide host range of livestock including bovine.

A total of 1034 cattle and buffaloes serum samples from West Bengal (93), Karnataka (94), Tamil Nadu (81), Odisha (98) and Madhya Pradesh (668) were screened by ELISA. An overall 51.06 % samples were found positive for the presence of antibodies of surra (Fig. 11).

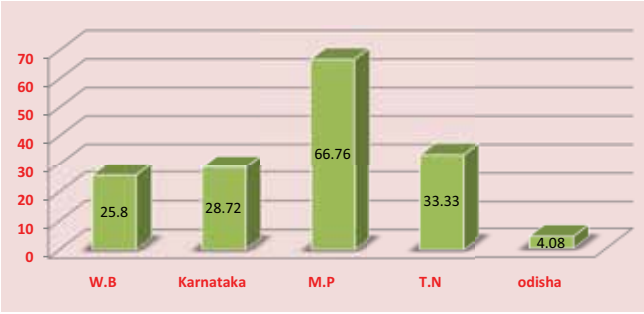

Fig 11. Surra antibodies in bovines

A total of 626 serum samples were collected from camels in different districts of Rajasthan including Jaipur, Hanumangarh, Udaipur , Bikaner, Ajmer, Barmer and Jaisalmer were screened by ELISA for the presence of antibodies against surra. An overall, 21.4% (134) sera samples were found positive for antibodies of *T. evansi* by ELISA (Fig. 12). The samples from Jaipur showed 32% positive, followed by Jaisalmer (24%), Udaipur (22%), Ajmer (21%), Barmer (20%), Bikaner (19%) and Hanumangarh (13%).

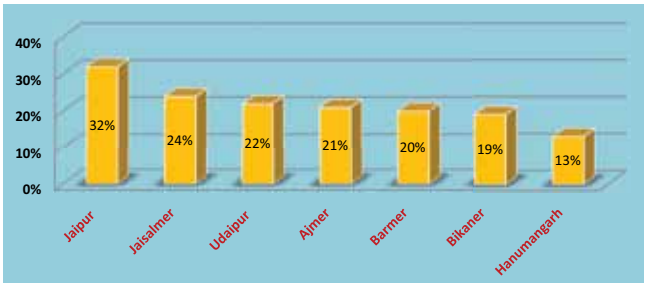

Fig 12. Surra antibodies in camels

Blood samples collected from 70 camels were subjected to PCR and ELISA for detecting organism

and antibodies. 35.7% and 45.7 % were found positive respectively in PCR and ELISA.

Faecal Sample Examination

A total of 304 faecal samples were collected from cattle, buffalo, sheep and goats from Karnataka, West Bengal and Odisha. Overall 37% of the samples were found positive, of which *Fasciola* was 13%, *Strongyles* 17%, *Amphistomiasis* 7%.

Snail Tissue Examination

134 Snails (*Lymnea spp.*) were collected from West Bengal and Karnataka, of which 38% were found positive for the presence of *Fasciola* infection, when tested by PCR (W.B - 36% and Karnataka-61%).

Secondary data analysis

The analysis of time series data (1990-2012) available at NADRES, revealed that there was a post monsoon (Aug. - Sept.) peak in outbreak of fascioliasis among goats in the country (Fig. 13). This incident coincides with the post breeding season of snail hosts. In cattle, two peaks were observed in the incidence of fascioliasis, one in pre-monsoon (April - June) and another in post-monsoon (Aug - Nov.). During last 22 years, there were two peak in the outbreak of trypanosomosis in buffaloes were observed – one in pre-monsoon and another in post monsoon (coincides with the breeding season of tabanid fly vector).

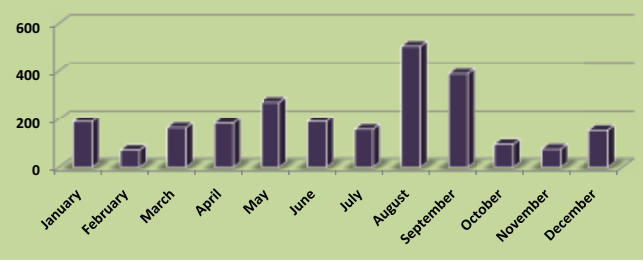

Fig 13. Month-wise Prevalence of Fascioliosis in Goat during 1990-2012

Inter Institutional Projects

IPC: ANSCNIVEDICOL201300400047 Project ID: OXX02385

Assessment of Socio-Economic Impact of FMD and its Control in India

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B.B.Dash, S. Saravanan, S.S.Pawar, G.K.Sharma (PD-FMD)
B.Ganseh Kumar (NAARM); R.G.Bambal (DADF), J. Mishri (ICAR) and AICRP FMD center

India has 528 million FMD susceptible livestock population. The small holders and landless farmers together possess 75% of the country's livestock resource and earn nearly half of their income from it. In such a scenario, occurrence of FMD has severe negative impact on their livelihood. FMD causes huge loss not only to the animal owners but also to the nation as a whole due to ripple effect on the downstream and upstream stakeholders. It is pertinent to understand the impact of the disease at the farm level and communicate the same to the policy makers, so as to extend appropriate support to animal health issues.

The secondary data was collected, analyzed and mapped. The time series data on outbreak, attack, death and animal density was collected for different districts for each of the eleven identified states. The scaling technique and Linear Discriminant Analysis (LDA) was employed to classify the high, medium and low risk districts in each of the identified state. The risk map for Karnataka state is presented in Fig.14. In conclusion, the high, medium and low risk FMD districts were identified for important states of India and risk districts were maps is presented.

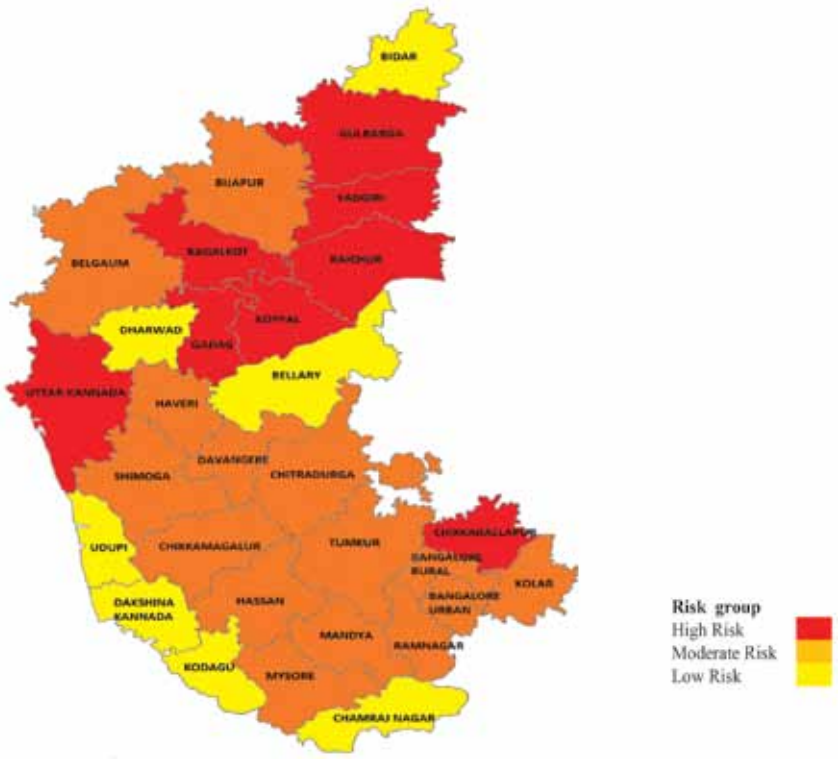


Fig. 14: FMD risk districts in Karnataka

IPC: ANSCNIVEDICOP201201100035 Project ID: IXX09659

Risk Analysis of Introduction of Notifiable Avian Influenza (NAI, HPNAI and LPNAI) in India with Special Reference to Risk of NAI through Trade and /or Non-trade Activities

K.P. Suresh (NIVEDI), D.D. Kulkarni , S. Bhatia, H.V. Murugkar, C. Tosh (HSADL, Bhopal)

Import risk analysis (IRA) is the process by which importing authorities determine whether live animal import or their products pose a threat to the animal and human population of their country. This is usually undertaken by the competent authority for the importing country, as well as by the individual who wants to import live animals to their farm or site. An import risk analysis involves the steps of hazard identification, risk assessment, risk management and risk communications.

Methodology was developed for assessing the risk of introduction of notifiable avian influenza (NAI: HPNA1 and LPNA1) in India. Risk analysis is dependent on the following epidemiological and disease characteristics. 1. Disease status of exporting country, 2. Nature of disease hosts, 3. Modes of transmission, 4. persistence of infection, and 5. Agent survival in animal products and by-products. Importing counties not only consider the risk assessment in import decision making but also assessment veterinary services, disease surveillance system, disease regionalization and zoning programs etc,.

The quantitative risk assessment consists of product of two probabilities

1. Probability of agent entry (PAE)

2. Probability of domestic exposure(PDE)

Risk assessment (RE)=PAE*PDE

PAE can be obtained using

PAE= 1-(1-CF1xCF2)nAIU, where CF1 is the country factor and CF2 is Commodity factor, AIU is the animal import unit, for example, one AIU can be 250 kg half-carcasses of beef, 50 kg of carcasses of pork or 5kg of frozen cattle liver etc as is defined by SME.

IPC: ANSCNIVEDISIL201300300046 Project ID: IXX10615

Retrospective Epidemiological Studies on HPAI with Reference to Spatio-Temporal Pattern and the Probable Associated Risk Factors Identification

R. Sridevi,, K. P. Suresh and P. Krishnamoorthy (NIVEDI), A. A. Raut,(HSADL, Bhopal)

Highly pathogenic avian influenza (HPAI) subtype H5N1 is a trans-boundary animal disease that has crossed the animal–human species barrier and over the past decade has had a considerable impact on the poultry industry, wild bird populations and on human health. Secondary data on outbreak was collected from different sources (affected population, susceptible birds population, population dead, places of outbreaks, GIS coordinates of the place of outbreak) from the year 2008 upto 2012 (additionally 2006, 2007 data also included) and compiled. The states affected with H5N1 AI outbreaks (from 2006-2012) were Maharashtra, Madhya Pradesh, Gujarat, Manipur, Assam, West

Bengal, Tripura, Sikkim, Odisha, Meghalaya, Karnataka (Fig. 15). Maximum number of outbreaks occured in West Bengal (55) followed by Assam (18). Among the various districts affected, Murshidabad in West Bengal reported 11 outbreaks followed by Kamrup in Assam with 8 outbreaks. 16 districts were affected out of 19 districts in West Bengal in 2008. Month wise analysis outbreaks revealed that the disease was observed in all months except in June. Case fatality rate was calculated for different states based on year wise data. CFR ranges from 22.5% - 100%. Morbidity and Mortality rates were calculated for different states for different years. Apparent Morbidity rates were ranged from 0.15% -93.05%. Apparent mortality rates were ranged from 0.15% -92.4%. Spatial map for H5N1 AI outbreaks were prepared. The map prepared depicted the number of outbreaks in different districts dividing them to very high, high, medium and low occurrences (Fig. 16). In conclusion, most of the H5N1 AI outbreaks occurred in North Eastern states of India. So far, West Bengal was the highly affected state. Outbreak frequencies were more during winter/cold season than any other seasons. Outbreaks were more common in January month which has very low temperature in most parts of India.

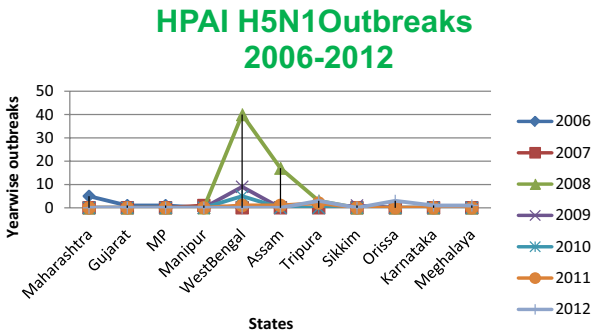


Fig.15: Graphical representation of H5N1 AI outbreaks in Indian Poultry in different states

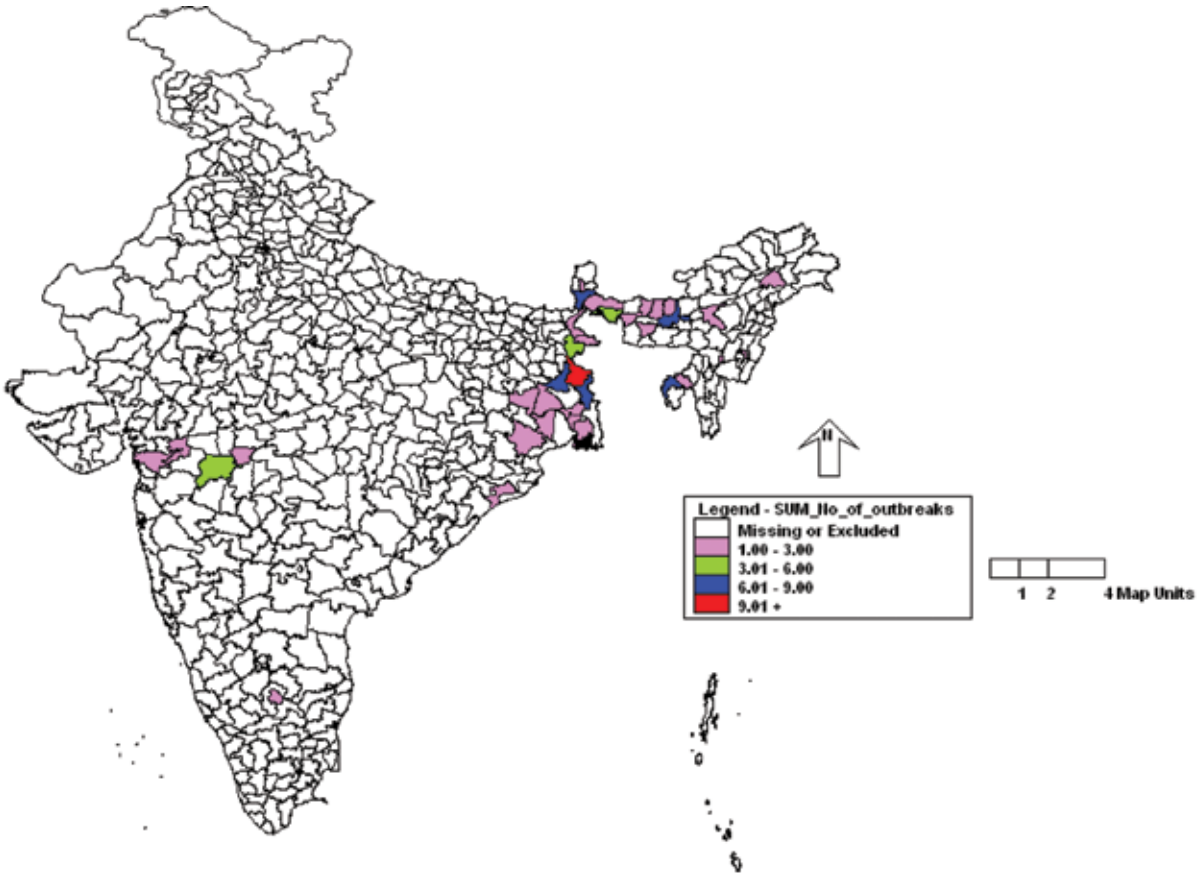


Fig. 16: Different districts of India affected by H5N1 outbreaks in poultry from 2006 - 2012

IPC: ANSCNIVEDICOP201201400038 Project ID: IXX10059

Cross-sectional Surveillance of Malignant Catarrhal Fever Infection in Domestic and Wild Ruminants in Southern India

D. Hemadri, K. P. Suresh, M. R. Gajendragad and S. S. Patil (NIVEDI)
Richa Sood, Victoria Chanu and Manoj Kumar (HSADL, Bhopal)

Malignant catarrhal fever (MCF) is a fatal lympho-proliferative disease of cattle and other ungulates caused by the herpes viruses of subfamily gamma-herpesvirinae belonging to the genus Macavirus. Presently, there are six members of the Macaviruses that are associated with clinical MCF. Alcelaphine herpes virus-1 (AlHV-1) is the first identified MCF virus, which persists as a subclinical infection in wildebeest (Connochaetes sp.) and is the causative agent of wildebeest-associated MCF. Clinically susceptible species such as cattle, bison, deer and pigs acquire the virus through inhalation or ingestion of virus-laden secretions from contaminated food stuffs or water.

Prevalence of OvHV-2 infection in sheep population of Karnataka

Prevalence of OvHV-2 infection in sheep population of Karnataka was calculated by at a confidence interval of 95%. Out of 356 samples screened from the state, 87 samples were positive for OvHV-2. The true prevalence of OvHV-2 infection in sheep of Karnataka was 24.44% (95% confidence interval, CI: 19.97 to 28.90).

Prevalence of OvHV-2 infection in different districts

Prevalence of OvHV-2 infection in sheep population of 11districts of Karnataka is presented in Table 9. The prevalence was highest in Raichur district and the lowest in Mandya.

Table 9: Prevalence of OvHV-2 infection in different districts

S.No.	District	True Prevalence	95% CI
1	Bagalkot	25.58	12.54-8.62
2	Belgaum	22.58	7.86-37.30
3	Bellary	16.13	3.18-29.08
4	Bijapur	18.75	0.00-37.87
5	Chikkaballapur	17.39	1.90-32.88
6	Chitradurga	25.86	14.59-7.13
7	Koppal	16	1.93-30.37
8	Mandya	14.29	0.00-29.25
9	Raichur	42.42	25.56-9.29
10	Tumkur	39.02	24.09-3.96
11	Yadgir	14.71	2.80-26.61

Prevalence of OvHV-2 infection in different taluks in Karnataka

Prevalence of OvHV-2 infection in sheep population of 22 taluks is presented in Table 10. Highest prevalence was observed in Raichur taluk. Raichur taluk borders Andhra state and interstate migration is a common practice and farmers from across the state are involved in animal exchanges and could be the reasons for relatively higher prevalence in that area. However, clear picture will emerge only after the survey of MCF in Andhra Pradesh. Maddur and Shorapur taluk have lots of lands under irrigation and grazing is not a problem in the dry season and as result migration is not a practice in these areas. Hence lower prevalence of the disease is expected and the migration which occurs to these places appears to have less impact on disease transmission due to non-mixing of local flocks and the migratory flocks. Surface map of OvHV-2 infection in Karnataka is given in Fig. 17.

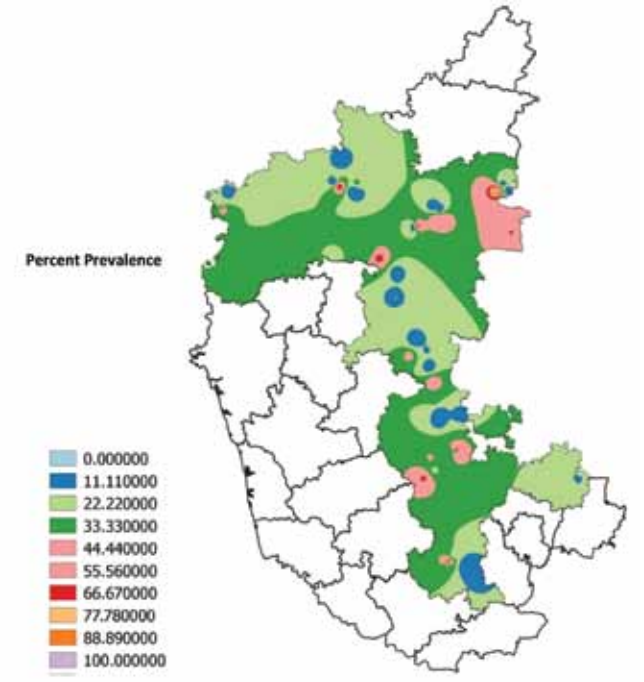


Fig. 17: Surface map of MCF prevalence

Age and sex wise prevalence of OvHV-2 infection

The observed prevalence in young animals (less than 1 year of age) and for adult animal (greater than 1 year of age) was 17.48% (95% CI: 10.14 to 24.81) and 27.27% (95% confidence interval, CI: 21.78 to 32.76) respectively. Since the virus is acquired after the birth, the lower prevalence in young animals is justified. The observed prevalence of OvHV-2 infection in ram and ewe was 35% (95% confidence interval, CI: 14.10 to 55.90) and 23.81% (95% confidence interval, CI: 19.26 to 28.36), respectively. Higher prevalence of rams is justified as these animals change flocks (unlike ewes) more frequently and have more chances of acquiring infection.

Table 10: Prevalence of OvHV-2 infection in different taluks

S. No.	Taluk	True Prevalence	95% CI
1	Badami	23.33	8.2-38.47
2	Bagevadi	12.5	0-35.42
3	Bijapur	25	0-55
4	Challakere	26.09	8.14-44.03
5	Chikballapur	18.18	0-40.97
6	Chiknayaka-halli	36.36	7.94-64.79
7	Chikodi	22.22	3.02-41.43
8	Chintamani	16.67	0-37.75
9	Gokak	23.08	0.17-45.98
10	Hiriyur	25.71	11.24-0.19
11	Hungund	30.77	5.68-55.86
12	Koppal	12.5	0-28.7
13	Kudligi	33.33	6.66-60
14	Kushtagi	22.22	0-49.38
15	Lingusugur	36.36	16.26-6.46
16	Muddur	0	0-34.81
17	Nagamangala	21.43	0-49.92
18	Raichur	54.55	25.12-3.97
19	Sandur	5.26	0-15.3
20	Shorapur	0	0-20.58
21	Sira	40	22.47-7.53
22	Yadgir	23.81	5.59-42.03

Molecular Phylogeny

The sequence analysis of tegument region OvHV-2 DNA products revealed high degree of similarity (99%) with the OvHV-2 reference sequence (Accession no. AY839756.1) and other sequences reported earlier from India (Fig. 18). The two sheep isolates showed a high degree of identity between them and these also matched closely with the bison isolate from Karnataka (Accession no. JQ801454). This clearly indicated that the bison got infected from the sheep. Phylogenetic analysis revealed that both sheep and bison isolate from Karnataka had maximum similarity with the South African cattle isolate (Accession no. EU851176).

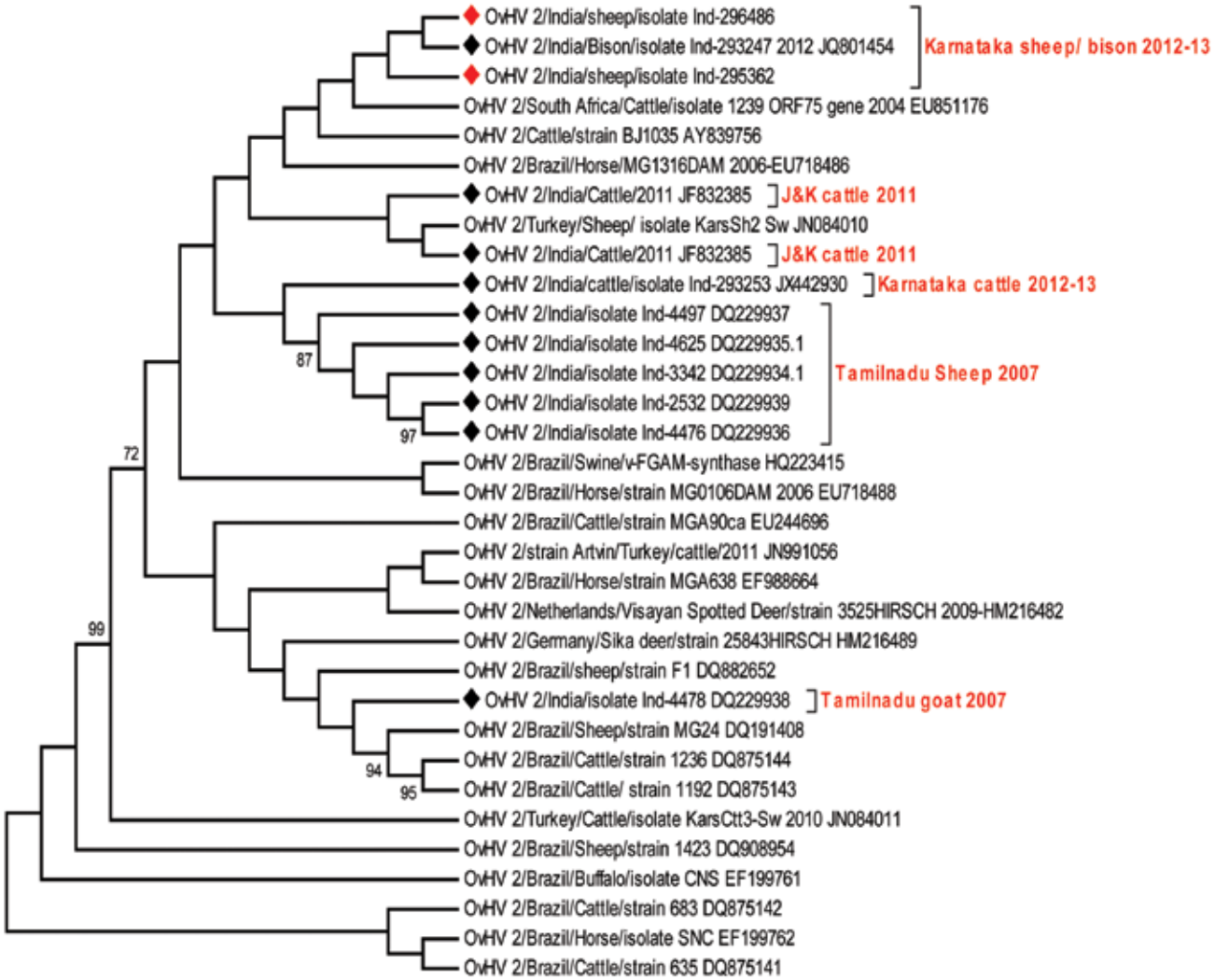


Fig. 18: Phylogentic analysis of OvHV-2 isolates

External Funded Projects

IPC: ANSCNIVEDISOP200900500017 Project ID: OXX02232

Outreach Programme on Zoonotic Diseases

R. Shome, V. Balamurugan and M. Nagalingam

A. Epidemiology of concurrent zoonotic diseases (*Leptospira, Brucella* and *Listeria*)

During the period under report, 295 serum samples (160 bovine, 19 sheep & goat, and116 human) were tested for antibodies against *Brucella*, *Leptospira* and *Listeria* by iELISA, Microscopic agglutination test and Listeriolysin-O based iELISA, respectively. High seropositivity was found with the *Leptospira* followed by *Brucella* and *Listeria* respectively. (Table 11). Prevalence of 6.1% of *Leptospira* and *Brucella* and 1% occurrence of all the three zoonotic diseases, clearly shows the concurrent occurrence of the multiple zoonotic diseases in livestock and human beings. Hence regular monitoring of the zoonotic diseases by sensitive diagnostic tests, good managemental practices in the farms, awareness campaigns are essential for control of the diseases.

Table 11: A differential analysis of seroprevalence for Brucellosis, Leptospirosis and Listeriosis in different livestock species

Species	Total samples analysed	Brucellosis*			Leptospirosis**			Listeriosis
		RBPT	ELISA	PCR	MAT	PCR	Isolations	LLO ELISA
Sheep	8	0	0	0	3	-	-	0
Goat	11	2	3	0	5	-	-	0
Human	116	21	26	9	25	2	-	0/13
Cattle	160	21	21	8	80	24	3	11
Total	295	44	50	17	113	26	3	11

**In case of Brucellosis, seropositive samples were further analysed with BCSP 31 PCR.*

*** Leptospirosis seropositive samples were anlysed with 16s rRNA PCR and Isolation.*

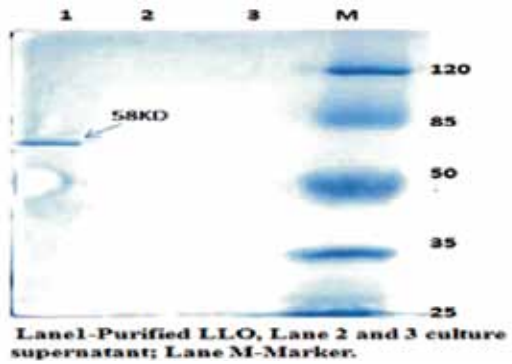
B. Listeriolysin-O based Lateral Flow Assay (LFA) development and evaluation:

Listeriolysin–O, a major virulence factor involved in pathogenesis was harvested from *L. monocytogenes* cultures grown at 37^{0}C for 16 hrs on brain heart infusion broth and cell free supernatant was precipitated by ammonium sulphate precipitation, purified by DEAE agarose anion exchange chromatography, tested by SDS PAGE/ Western Blot (Fig.19) and evaluated by iELISA with hyperimmune sera raised in rabbits. Lateral Flow assay works on immune - chromatographic Assay principle and has great potential as point of care diagnostic. Test sample flows through the adsorbent pad by capillary action and if sample contains anti Listeriolysin-O antibodies, two lines are visible indicating as positive.

Lateral Flow Assay kits for *Listeria* diagnosis was designed in collaboration with M/s Ubio Biotechnology systems Pvt Ltd., Cochin is working satisfactorily with serum samples of livestock and humans. LFA tests showed 100% agreement with LLO ELISA. But LFA test device failed to detect anti *Listeria* antibodies in blood sample analysis. Hence further improvement of the test device is required to make it suitable for blood samples also with greater sensitivity (Table 12).

Table 12: Evaluation of Listeria LFA

Sl. No.	Species	Total No. of Samples	LLO iELISA Positive	Serum LFA Positive	Blood LFA Positive
1	Cattle	170	11 (6.5%)	11(6.5%)	0
2	Sheep and Goat	119	7 (5.8%)	7 (5.8%)	0
3	Human	116	0	0	0
	Total	**405**	**18 (4.4%)**	**18 (4.4%)**	**0**



Lane1-Purified LLO, Lane 2 and 3 culture supernatant; Lane M-Marker.

Fig. 19: Detection of purified LLO in SDS-PAGE

C. Epidemiology and Risk analysis of human brucellosis

The total of 1175 human samples broadly classified in two groups based on their occupation like "risk group" (1112) consisting of veterinarians, para-veterinarians, farm workers, animal-handlers and farmers and "non-risk group" (63) which do not fall in the described category distributed in 7 groups based on age (in years). All the samples were screened for brucellosis by serology and PCR as shown in table 13. In non-risk population the prevalence was 9%.

Statistical analysis revealed that the 31-40 and 51-60 years age groups are more susceptible to contact brucellosis under both the groups of population and sex-wise analysis revealed that the males are more prone to contract brucellosis in comparison to females. This indicates the sex has significant role on the prevalence of brucellosis when tested with all the tests used in the study.

Table 13: Overall analysis of human brucellosis prevalence by various tests

Parameter		Cases (n)	Risk group			Non-risk group			$\chi 2$	P Value
			Positive	Negative	Total	Positive	Negative	Total		
Age	1-10	3	0	1	1	1	1	2	0.19	0.663
	11-20	23	0	11	11	3	9	12	1.34	0.247
	21-30	179	10	148	158	4	17	21	2.58	0.108
	31-40	652	29	603	632	6	14	20	19.89	<0.001**
	41-50	205	8	186	194	2	9	11	1.92	0.166
	51-60	45	1	38	39	2	4	6	3.74	0.054*
	>60	5	0	3	3	0	2	2	-	-
	Un-grouped	63	0	0	0	9	54	63	-	-
Gender	Male	1066	48	932	980	20	66	86	41.59	<0.001**
	Female	109	0	58	58	6	45	51	5.14	0.023*

***Highly significant; *Significant. Risk group includes veterinary officers, animal handlers, para veterinarians. Non-risk group includes those who are not in direct touch with animals like house wives*

D. Human neurobrucellosis

Neurobrucellosis, a subtle disease, is uncommon with heterogeneous clinical profile. Hence, high degree of suspicion and recognition is prudent and fundamental for accurate diagnosis and management. Abnormal cerebrospinal fluid (CSF) profile is just a sign a disease, hence laboratory confirmation and detection of different (active versus chronic) phases of the disease always requires laboratory confirmation. A total of 80 suspected human neurobrucellosis cases were tested by RBPT, IgM and IgG ELISA and BCSP 31 genus specific PCR. According to this study 21-30 and 31-40 years age groups are more susceptible to neurobrucellosis (Table 14). This study also indicated that IgM ELISA is very essential to state the active infection status and combination of serological and PCR tests confirm infection status for monitoring the treatment.

Table 14: Human neurobrucellosis case evaluation

Age group	Total Subjects analyzed	RBPT	IgM ELISA	IgG ELISA	PCR	Status
1-10	2	1	1	1	0	1
11-20	5	1	0	1	0	1
21-30	16	3	4	3	2	4
31-40	31	3	3	3	3	3
41-50	19	2	1	2	2	2
51-60	5	1	1	1	1	1
61-70	2	-	-	-	-	
TOTAL	**80**	**11(13.75%)**	**10(12.5%)**	**11(13.75%)**	**8(10%)**	**12(15%)**

IPC: ANSCNIVEDICOL201201500039 Project ID:OXX02733

DBT-Network Project on Brucellosis: Project Monitoring Unit

H. Rahman and G.B. Manjunatha Reddy

The project monitoring unit under DBT-Network project on brucellosis involved in coordination of different activities like; the recruitment of manpower in the network, monitoring the effective utilization of funds under the non-recurring head, procurement of antigens, kits, standard cultures and sera and other biologicals required for the project, monitoring progress at different centers, preparation and submission of audited statements, auditing of financial accounts on an annual basis for claim from DBT on behalf of the network, designing, updating and maintenance of website for the project. The PMU has organized one day training cum midterm review meeting for all project investigators at NIVEDI, Bengaluru, on 1st June 2013 and a three day training programme entitled "Hands-on Training on Laboratory Techniques in *Brucella* Research" was organized at NIVEDI, Bengaluru from 1st – 3rd, August, 2013 for contractual personnel working under different sub-centers. Organized annual review meet at Ludhiana from 29th -30th October, 2013. Submitted monthly, quarterly and annual reports to DBT and also published annual report on behalf of DBT. Co-ordinated a training at Madurai Kamaraj University on "*Brucella* Genomics" from 8th – 12th July, 2013 and 2 day workshop on "Isolation of Brucella" from 13th-14th March, 2014 at Izatnagar, Bareilly.

IPC: ANSCNIVEDISOP201201600040 Project ID: OXX02578

DBT Network Project on Brucellosis: Brucellosis Epidemiology

R. Shome, B. R. Shome and M. Nagalingam

A. Comparative evaluation of serological vis-a-vis PCR assay for diagnosis of brucellosis in cattle.

A total of 1171 cattle samples (Male 24, Female 1147) were collected from 19 different organized farms from Karnataka, Goa and Andhra Pradesh states. Out of 1171 samples, 70(5.09%) were detected positive by RBPT, iELISA revealed 88(7.50%) samples positive, bcsp31 serum PCR and bcsp31 blood PCR showed positive of 72 (6.14%) and 53 (4.52%) samples, respectively (Table 15). Comparative evaluation of tests revealed that 51(4.35%) cases were detected positive both in serum PCR and serology. It was noted that 7(0.59%) cases were solely detected by serum PCR which were declared negative by serology. Breed-wise comparison of the results revealed that, highest positivity of 42.18% (27/64) was recorded in buffaloes, followed by Holstein Friesian crosses 14(6.14%)and 9 (1.56%) in Holstein Friesian breeds. Indian breeds like Ongole, Sahiwal Cross, Hallikar Cross, and non-dscriptive breeds were free of brucellosis (Table 16 and Fig. 20). Present study concluded that it is very difficult to detect all infected cases of bovine brucellosis using a single test but combination of two tests are useful for diagnosis. Among PCR, serum PCR seems to be promising assay as it detected highest numbers of positive cases.

Table 15: Comparative analysis of bcsp 31 serum and blood PCR with serological tests (RBPT and IELISA)

	Total No.of Samples	RBPT + ve only	IELISA +ve only	Serum PCR +ve only	Blood PCR +ve
Male	24	1(4.16%)	4(16.7%)	2 (8.34%)	1(4.16%)
Female	1147	69(6.01%)	84(7.31%)	70(6.13%)	52(4.5%)

Table 16: Breed-wise analysis of prevalence of brucellosis

Sl. No.	Breed	Total No. of Samples	RBPT + ve	IELISA +ve	Serum PCR +ve	Blood PCR +ve
1	HF	574	16 (2.78%)	21 (3.65%)	16 (2.78%)	9 (1.56%)
2	HF Cross	164	10 (6.09%)	16 (9.75%)	18 (10.97%)	14 (6.14%)
3	Jersey	228	10 (4.38%)	11 (4.8%)	1 (0.44%)	1 (0.44%)
4	Jersey Cross	17	0	2 (11.76%)	1 (5.9%)	1 (5.9%)
5	Murrah	64	33 (51.56%)	34 (53.12)	34(53.12)	27 (42.18%)
6	Gir Bull	24	1 (4.16%)	4 (16.7%)	2 (8.34%)	1 (4.16%)
7	Ongole	62	0	0	0	0
8	Sahiwal Cross	1	0	0	0	0
9	Hallikar Cross	3	0	0	0	0
10	Non descrip-tive breeds	34	0	0	0	0
	Total	**1171**	**70(5.97%)**	**88(7.51%)**	**72(6.15%)**	**53(4.53%)**

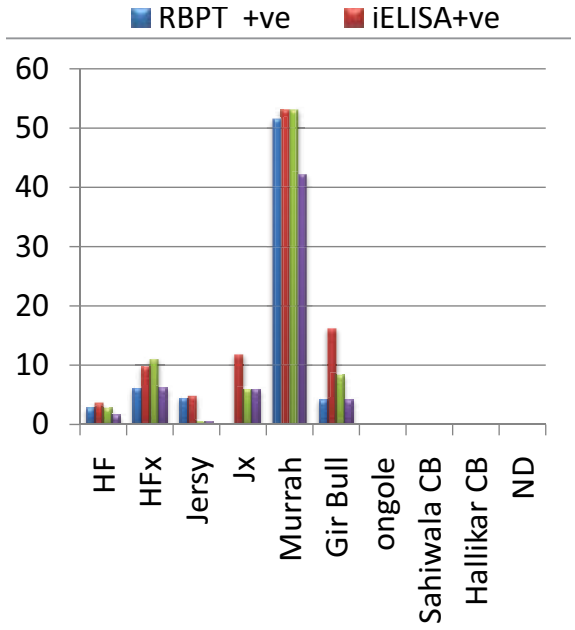


Fig 20: Breed wise brucellosis prevalence

B. Evaluation of milk versus serum for diagnosis of brucellosis

The *Brucella* antibody levels in milk and serum of cattle were determined by Milk Ring Test (MRT), milk ELISA, RBPT and serum ELISA. Undiluted milk samples and 1:100 diluted serum samples used in Protein G based indirect ELISA. Milk and serum samples collected from 298 cattle, were examined for the diagnosis of *Brucella* antibodies by (MRT), milk-ELISA, RBPT and serum-ELISA. Of these, 22 (7.38%), 18(6.04%), 21 (7.04%) and 30 (10.06%) were found to be positive by MRT, milk ELISA, RBPT and serum ELISA respectively. Only 5(1.67%) and 27 (9.06%) were positive by genus PCR using milk and serum DNA respectively (Table 17). Even though good correlation has been found in between MRT and RBPT, the antibody levels were about 5–10 times higher in serum than milk in majority of the samples tested. Even in some of the serum PCR positive animals also, milk samples showed negative for *Brucella* antibodies. Based on these observations, it is reasonable to conclude that the serum is most preferred material than milk for diagnosis of brucellosis.

Table 17: Comparative analysis of Milk versus Serum

		MILK			SERUM		
Breeds	**Total No. of Samples**	**MRT**	**iELISA**	**PCR**	**RBPT**	**iELISA**	**PCR**
HF	164	9 (5.48%)	9 (5.4%)	3 (1.8%)	11 (6.7%)	14 (8.5%)	12 (7.3%)
HF Cross	121	13 (10.75%)	9 (7.4%)	2 (1.65%)	9 (7.43%)	15 (12.3%)	14 (11.5%)
Jersey	13	0	0	0	1 (7.6%)	1 (7.6%)	1 (7.6%)
Total	298	22 (7.38%)	18 (6.04%)	5 (1.67%)	21 (7.04%)	30 (10.06%)	27 (9.06%)

C. Speciation of *Brucella* directly in clinical samples

Hinic PCR assay was standardized for rapid detection of the *Brucella* genus and for the differentiation of *Brucella species* viz., in clinical samples by genus specific IS711 primer (63bp). In case of species specific Hinic PCR, *B. melitensis* amplified 67bp, 81bp and 83bp amplicons suggest *B. abortus* and *B. suis*, respectively (Fig. 21). Two human samples which were positive by RBPT & ELISA were confirmed by genus & species specific Hinic PCR as *B. abortus*. The advantage of Hinic PCR is speciation of *Brucella* species directly in the clinical samples because of less amplicon size and another advantage is same primers can be used for gel based and real time PCRs. Hence, Hinic PCR serves as an excellent tool for diagnosis of brucellosis in clinical samples.

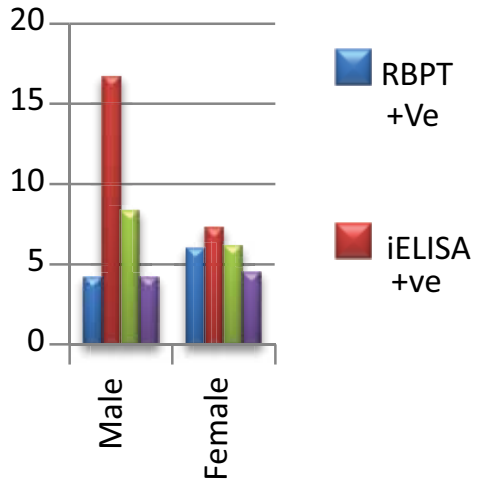


Fig. 21: Sex-wise prevalence of brucellosis

D. Salient features of full genome sequence of *Brucella*

Whole genome shortgun sequencing has been completed for one each of field isolate and reference strain and genome sequence are available in NCBI GenBank.

a) *B. melitensis* ADMAS G1 (field isolate): Accession no: NZ_AUTT00000000

b) *B. abortus* ADMAS S99 (reference strain): Accession no: AWTU00000000.

In ADMAS G1 (field isolate): A total of 3,388 genes were predicted, of which 3,325 are protein-coding genes. Overall, 2,610 of the protein-coding genes were annotated with putative functions and 715 genes were annotated as hypothetical proteins. RAST predicted 63 RNA genes, of which 57 were tRNA and 6 were rRNA genes. A total of 58 genes were predicted to be involved in the pathways responsible for virulence mechanisms, such as the virB type III, IV, and V secretory pathways and the sec-independent protein secretion pathway component TatC, etc. In addition, 44 defense mechanism genes, which include the genes responsible for the ABC-type multidrug transport system, multidrug resistance efflux pump, restriction endonuclease etc., were also identified. Like other *Brucella* spp., this strain contains genes responsible for flagellum formation. But it lacks genes responsible for the chemotactic system that is necessary for assembling a functional flagellum.

E. Multiple locus sequence typing of *Brucella* (MLST)

The raw sequence data of four reference strains and 5 field isolates were edited using ChromasLite 2.01 software. The MLST sequences (accession number from AM694191 through AM695630) of the strains described by Whatmore et al. (2007) were downloaded from GenBank database. Each distinct allele at each of the nine loci was given a numerical designation according to sequence of defined alleles. Each unique allelic pattern over all loci was identified as a sequence type (ST). The sequence type of reference strain *B. Abortus* S99 was found to be ST1, *B. abortus* S19 to be ST 5, *B. melitensis* 16M is ST 7 and *B. suis* 1330 is ST14. All the *B. abortus* field isolates were carrying ST1 which is similar to standard strain *B. abortus* S99. The one *B. melitensis* field isolate is carrying ST7 similar to ST of *B. melitensis*16M. Phylogenetic tree construction and GC analysis are shown in Fig. 22.

Overall 4 distinct Sequence Type (STs) was identified among the 4 standard and 5 field *Brucella* isolates. The relationship between STs were examined by constructing a neighbour joining tree from the concatenated nucleotide sequences of 4,396bp spanning all 9 loci DNA fragments that comprised each ST. The phylogenetic analysis was made between the Indian isolates and few global *Brucella* isolates. All the isolates has fallen under two different clusters. Cluster 1 comprises of all the Indian and global *B. abortus* isolates in one clade and all *B. melitensis* isolates in clade 2. Whereas the cluster 2 includes all other species of *Brucella* species viz., *B. microti, B. ceti, B. pinnipedialis, B. ovis, B. suis and B. canis* under different clades. But among the different *Brucella* species, same species are falling under same sub-clades. Thus shows the genetic relatedness between the field and reference strain of different species. Based on the phylogenetic analysis, we could confirm the genetic relatedness between the Indian and Global *Brucella* strains.

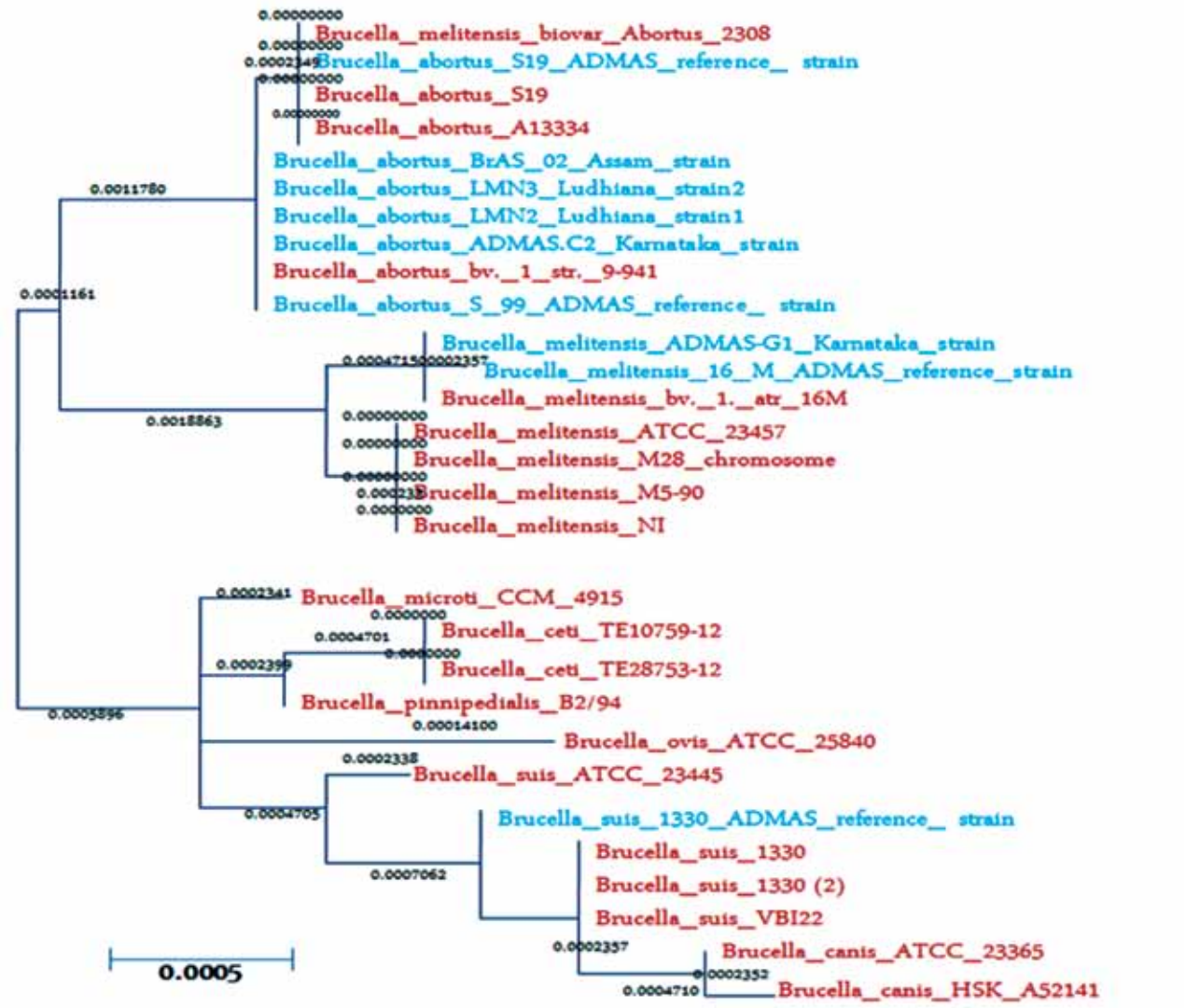


Fig. 22: Phylogenetic tree for *Brucella* isolates using MEGA software version 4.0

F. *Brucella* specific Real Time PCR

A total of 5 probe based monoplex Real-time PCR was performed for Internal Positive Control (IPC)-puc19, *Brucella* genus and *Brucella* species (*B. abortus, B. mellitensis* and *B. suis*).The reactions were set using specific primer probe in a final volume of 20 μl, containing 2× master mix, forward and reverse primers (2.5 picolmoles), FAM, HEX and Cy5-labeled probes(5 μM), and template DNA (2 μl). The cycling parameters consisted of incubation for 7 min at 95°C to activate enzymes and denature DNA, followed by 40 cycles of 95°C for 30 s and 60°C for 30 s, followed by a cooling step at 40°C for 30s. All other (genus and species) reactions worked successfully and specifically as shown in the Fig 23.

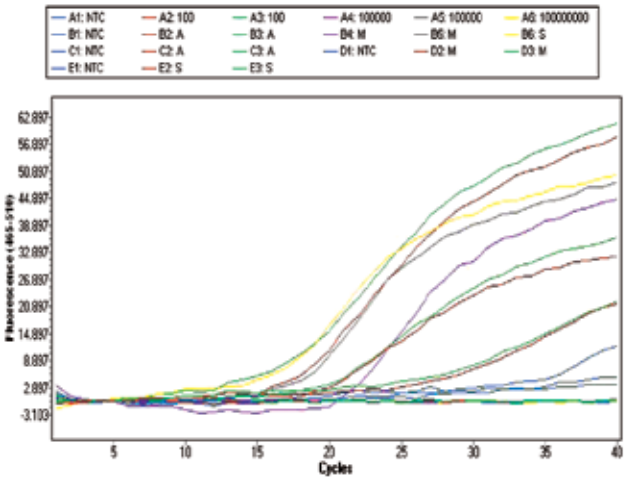


Fig. 23: Amplification curves in Real time PCR

IPC: ANSCNIVEDISOP201201700041 Project ID: OXX02384

DBT Network Project on Brucellosis: Brucellosis Diagnostics

M. Nagalingam, R. Shome, V. Balamurugan and G.B. Manjunatha Reddy

Brucellosis is a zoonosis of both public health and economic importance. The development of diagnostic tests based on the recombinant protein for detection of antibodies directed to *Brucella* proteins could help to circumvent these problems to some extent. So far, four genes (*sodC, bp26, p39* and *bls*) were amplified from *B. abortus* S99 strain, TA cloning performed in pGEMT easy vector in Top 10 F' cells and sequencing were carried out.

Cloning in the expression vector

The inserts were sub-cloned into pET32a vector at *EcoRI* and *NotI sites*. The *E.coli* Top 10F' cells were transformed with the ligated mixture and the transformants were screened by ampicillin selection. The efficiency of transformation was 2 x $10^4/\mu g$ of DNA. In order to express BLS protein, BL21 strain of *E.coli* was used. *E.coli* BL21 (DE3, lysogen and codon plus) cells were transformed with recombinant plasmids and grown on LB agar plates containing ampicillin (50μg/ml) at 37°C overnight.

Expression and characterization of recombinant proteins

The transformed BL21 colonies were then screened for the presence of expressed protein. For that, individual colonies were grown in LB broth containing ampicillin (50μg/ml) at 37°C till the culture reached mid log phase or obtaining an OD600nm of 0.4 – 0.5. The expression was induced at 30°C using 1mM IPTG. At this temperature, there would be increased expression and activity of a number of *E.coli* chaperons, thereby enhanced protein folding and reduced precipitation of inclusion bodies. Pre-induction incubation for 3 hr at 37°C was necessary to achieve mid-log phase growth. Samples were collected at 6h post induction and were analyzed in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as per standard protocols (Fig. 24). Then it was analyzed using anti histidine HRPO conjugate in western blot.

Similar strategy has been adopted for *bp26* and *sodC* gene to express *bp26* and sodC protein. The expression of *p39* gene is in progress. The expressed protein size matches with the expected size for BLS (~36 KDa) and bp26 (~44 KDa) protein but for the SodC, it (~17 KDa) differs from expected size (38 K Da). Hence it needs to be analyzed whether protein is cleaved because of the presence of signal protein. Further purification of recombinant BLS and BP26 proteins were standardized using Ni-NTA column. Standardization of ELISA using these proteins for diagnosis of brucellosis is in progress.

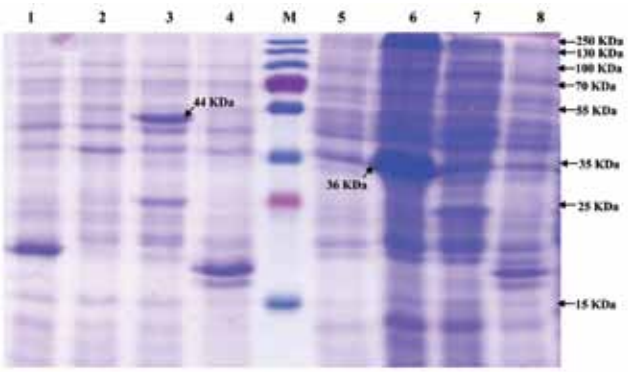


Fig. 24: SDS-PAGE profile of recombinant *Brucella* proteins

Lane 1 - Vector pET 32 (a) + codon plus BL21 host cell control; Lane 2 - Bacterial cell lysate from recombinant BLS protein clone in codon plus BL21 host cell; Lane 3 - Bacterial cell lysate from recombinant bp26 protein clone in codon plus BL21 host cell; Lane 4 - Bacterial cell lysate from recombinant SodC protein clone in codon plus BL21 host cell; Lane M-Marker; Lane 5- BL21(DE3) host cell control; Lane 6 - Bacterial cell lysate from recombinant BLS protein clone in BL21 (DE3) host cell; Lane 7- Bacterial cell lysate from recombinant bp26 protein clone in BL21 (DE3) host cell; Lane 8- Bacterial cell lysate from recombinant SodC protein clone in BL21 (DE3) host cell

IPC: ANSCNIVEDICOP201201300037 Project ID: OXX02226

Toll-like Receptors in Farm Animals - Evolutionary Lineages and Application in Disease Resistance

B. R. Shome

Milk sample collection, SCC measurement and mPCR (two tube) detection of major ten mastitis pathogens

Milk samples were collected from all lactating animals (Deoni n = 25) at NDRI at intervals (n=7 times) and similarly from HF purebred (n=74) at CCBF, Hessarghata at intervals (n=3 times). Somatic cell count was recorded for all using Nucleocounter SCC 100. Somatic cell count at regular time interval allowed categorizing of animals under two major groups viz., apparently healthy (>10,000 to 2,00,000 SCC/ml) and subclinical mastitic (>2,00,000 SCC/ml) groups (Fig 25 and 26).

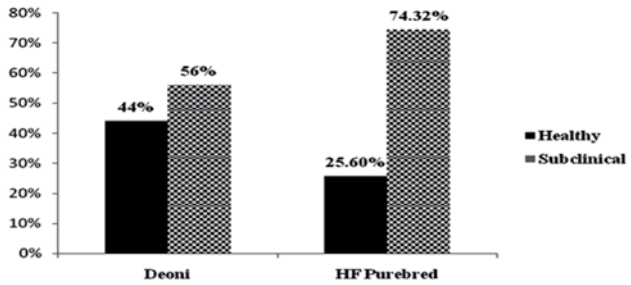


Fig. 25: Distribution of milch animals into Subclinical mastitis and Healthy on the basis of Somatic cell count cut off 2,00,000 SCC/ml

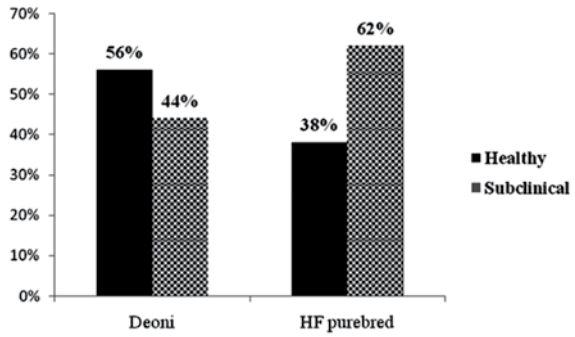


Fig. 26: Distribution of milch animals into Subclinical mastitis and Healthy on the basis of Somatic cell count cut off 5,00,000 SCC/ml.

The Deoni as well HF purebred samples showed prevalence of *Staphylococcus* with *S. aureus* being the most predominant (Fig. 27). HF samples showed prevalence of *Streptococcus* species followed by *Streptococcus* spp and *E. coli*. The percent prevalence of organism in subclinical versus healthy groups of Deoni is depicted in Fig. 28. *Streptococcus* spp was detected only in subclinical mastitis group of HF purebred samples only. No *Streptococcus* spp. was detected in Deoni milk samples (Fig.29).

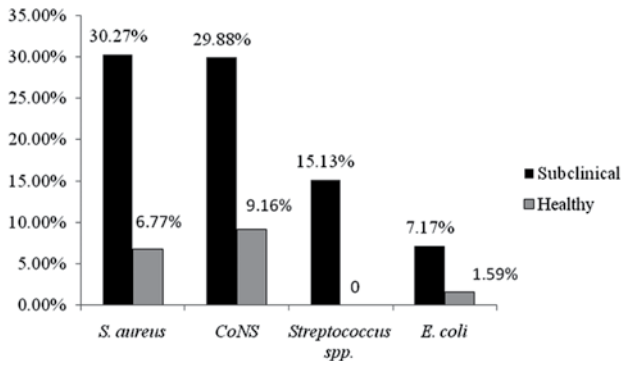


Fig 27: Percent prevalence of major mastitis pathogens in HF Purebred (Healthy vs Subclinical) using multiplex PCR

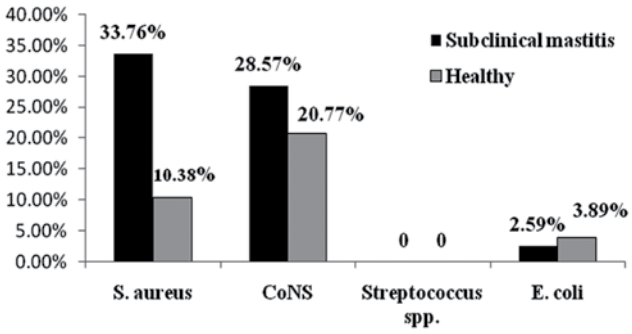


Fig. 28: Percent prevalence of major mastitis pathogens in Deoni (Healthy vs Subclinical) using multiplex PCR

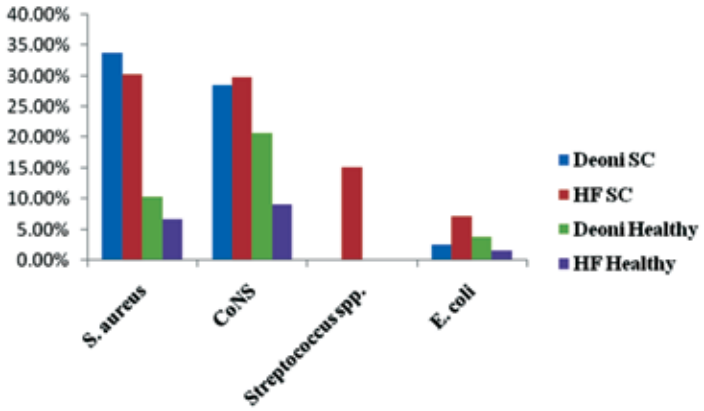


Fig. 29: Distribution pattern of organisms in Subclinical and Healthy group in both Deoni and HF samples

Polymorphism in promoter and exons of TLR2 and TLR4 genes in Healthy (H) and Subclinical mastitis (SCM) in two cattle breeds HF purebred (Bos taurus) and Deoni (Bos indicus)

Whole Blood was collected from 13 (7H + 6 SCM) Deoni (*Bos indicus*) and 21 (6H+15 SCM) HF purebred (*Bos taurus*) cattle breeds. Genomic DNA isolation was performed utilizing Qiagen DNA isolation kit. The 13 DNA samples representing Deoni (Bos indicus) and 21 DNA samples representing HF purebred (*Bos taurus*) cattle breeds were utilized for PCR Amplification and subsequent sequencing analysis of TLR2 and TLR4 genes. A total of 8 primer sets covering entire TLR2 gene exonic regions and 9 primer sets covering entire TLR4 gene exonic regions were used for PCR amplification. All the PCR Products comprising of TLR2 (238) and TLR4 (306) gene fragments were forward and reverse sequenced for SNP analysis in the two studied cattle breed populations. The ClustalW multiple sequence alignment of complete exonic regions of TLR2 gene in both the breeds revealed an overall of 23 SNPs, of which 19 SNPs were unique to Deoni breed and 3 SNPs were unique to HF purebred and 1 SNP was shared among the two studied breeds, respectively (Table 18).

Table 18: Amino acid substitutions observed in TLR2 gene for Deoni and HF purebred cattle breeds.

Sl. No.	Amino Acid Position	Variation	Breeds / Animal ID	Status Groups
1	63	D/E	Deoni/HF(D349, D433, D542, D560, H933 & H999)	SCM/Healthy
2	68	G/S	Deoni (D208, D311, D313, D329, D397, D469, D534, D549 & D552)	SCM/Healthy
3	152	R/Q	HF (H933)	Healthy
4	201	S/N	HF (H933)	Healthy
5	227	F/L	Deoni (D208, D311, D313, D329, D397, D534, D549, D552)	SCM/Healthy
6	326	H/Q	Deoni (D208-D560 13 Samples)	SCM/Healthy
7	337	R/K	Deoni (D208-D560 13 Samples)	SCM/Healthy
8	417	N/S	Deoni (D208-D560 13 Samples)	SCM/Healthy
9	563	R/H	Deoni (D208-D560 13 Samples)	SCM/Healthy
10	605	T/M	Deoni (D311, D313, D397, D534, D549 & D552)	SCM/Healthy
11	665	H/Q	Deoni (D208-D560 13 Samples)	SCM/Healthy

The PolyPhen-2 analysis utilizing the amino acid substitutions observed in the TLR2 gene revealed that the amino acid variation R/H at position 563 have a potential influence on the TLR2 gene 3D protein structure. This mutation is predicted to be possibly damaging with a score of 0.906 (sensitivity: 0.82; specificity: 0.94).

In TLR2 gene one GT nucleotides deletion in the GT repeats region (SINE's ART2A), was observed in the 5′ UTR region of the presently studied indigenous Deoni population only. The Phylogenetic analysis of the TLR2 gene among the Deoni and HF Purebred population revealed close clustering of breed specific sequences, but a clear demarcation between the two breeds was not observed among the two breeds studied (Fig.30).

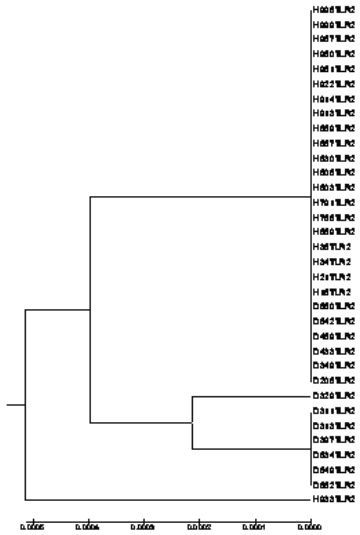


Fig. 30: UPGMA tree showing evolutional relationship between the Deoni and HF purebred based on the TLR2 gene sequences

Table 19: Samples categorized based on SCC/ml (Deoni)

	Subsets	ID	Collection 1	Collection 2	Collection 3	Collection 4	Collection 5	Collection 6	Collection 7
Sub clinical	D313	SC1	>20,000,00	>20,000,00	11,68,000	88,000	541000	2,90,000	3,50,000
	D412	SC2	7,15,000	1886000	936000	1473000	384000	236000	114000
	D418	SC3	5,46,000	293000	74000				
	D542	SC4	5,66,000	>20,000,00	17000	20000	<10,000	17000	59000
	D547	SC5	5,66,000	>20,000,00	17000	20000	<10,000	17000	59000
Healthy	D329	H1	1,01,000	56000	126000	14000	18000	12000	22000
	D377	H2	26,000	14000	19000	16000	23000	13,000	
	D534	H3	53,000	96000	<10,000	26000	13000	29000	27000
	D549	H4	76,000	25000	15000	19000	13000	13000	23000
	D555	H5	1,60,000	124000	81000	36000			

The ClustalW multiple sequence alignment of complete exonic regions of the TLR4 gene revealed an overall of 8 SNPs in Deoni and HF Purebred cattle breeds studied, of which 5 SNPs were unique to Deoni and 1 SNP was unique to HF Purebred and 2 SNPs were shared among the two studied breeds.

Comparative transcript levels for selected PRR and cytokines in milk somatic cells in healthy and subclinical mastitis groups from Deoni cattle breed

A total of ten samples (Deoni) comprising 5 from subclinical mastitis group and 5 from healthy group were selected (Table 19) for comparative transcript level for the selected PRR (TLR2 and TLR 4) and inflammatory mediators (IL6, IL10, IL12, TNF-α, IL-1β, IFN-α and IL8) in the milk somatic cells. Total RNA was isolated from milk somatic cells using RNAeasy mini kit (Qiagen) as per manufacturer's instruction. RNA samples were stored at -80°C till further use. The quality of the RNA was assessed by agarose gel electrophoresis (2.2 M formaldehyde) and ethidium bromide staining. The concentration of RNA was assessed by using Nanodrop 2000c.

The quantification of relative mRNA concentrations by quantitative real-time PCR (qRT-PCR) of selected genes was performed using gene specific primers

(Table 19) and probes from Universal Probe Library (Roche) using Roche Lightcycler® 480 real-time PCR system.

Relative quantification showed variation in mRNA levels between subclinical versus healthy groups. The fold difference was < 2 for all the genes studied except for the TLR2 gene. The PRR, TLR2 and interleukin IL8 were highly expressed in Subclinical mastitis group compared to Healthy group of Deoni samples. However, IL6 the key proinflammatory cytokine was found to be comparatively less expressed in subclinical group when compared to healthy group of Deoni samples. For the other inflammatory mediators (IL10, IL12, TNFα, IFNα, IL1β and TLR4), no distinguishable changes were observed in the expression levels between the subclinical and healthy groups.

Comparative transcript levels for selected PRR and cytokines in in-vitro cultured PBMCs upon LPS and LTA antigen stimulation in Healthy and subclinical mastitis sample groups from Deoni cattle breed

Whole blood was collected in Heparinized vacutainer from 5 Subclinical mastitis group and 5 from healthy group of Deoni (*Bos indicus*) cattle breed. The isolated PBMCs were grown overnight in RPMI 1640 media. Based on the cell count by haemocytometer, $1x10^6$ cells were distributed in each well in a 6 well cell culture plate and stimulated with LPS (100ng/well) and LTA (1μg/well) incubated and harvested at 2hr, 8hr and 24hr time points respectively. Quantitative real-time PCR was preformed for the 8 genes namely IL-1β, IL- 6, IL- 8, IL- 10, IL-12, TLR 2, TLR 4 and TNF- α using LC480 Probe master kit (Roche) along with 3 μg cDNA, gene specific primers and probes. To determine test gene mRNA levels, the gene copy numbers was normalized to that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) using 2-Ct inbuilt algorithim in the LC480 system.

Relative gene expression quantification analysis of cultured PBMCs upon LPS stimulation revealed inflammatory mediators IL1-β, IL6 and IL10 being up-regulated until 8hr time point and then expression declined progressively in both healthy and sub-clinical mastitis groups. The TLR2 and TLR4 Pathogen Recognition Receptors showed highest expression levels at 24hrs time point in both groups (Fig 31).

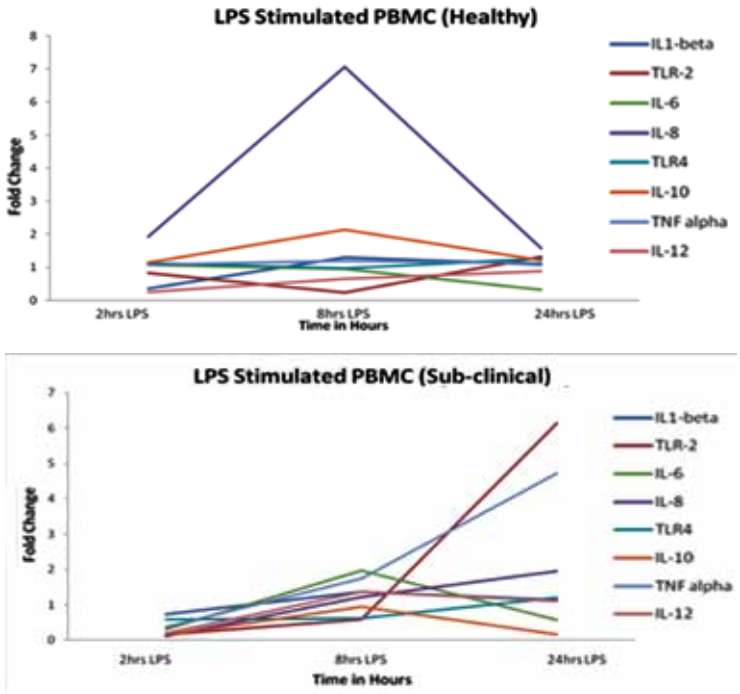


Fig.31: LPS stimulation of PBMC-Fold change in expression levels observed for selected PRR (TLR2 and TLR 4) and inflammatory mediators (IL6, IL8, IL10, IL12, TNFα and IL1β) in PBMCs in in-vitro culture upon LPS stimulation in Healthy and Subclinical mastitis sample group.

Relative gene expression quantification analysis of cultured PBMCs upon LTA stimulation revealed IL1-β, IL-6 and IL-12 genes were up-regulated until 8hr and then the expression lowered in both healthy and subclinical group. The TLR2 and TLR4 Pathogen Recognition Receptors showed highest expression levels at 24hr time point in both groups (Fig.32).

In contrast, in both LPS and LTA stimulated cultured PBMCs from healthy group revealed the pro-inflammatory cytokine IL-6 expression was highest at 2hr time point, whereas in subclinical mastitis group, the IL-6 expression was highest at 8hr time point (Fig. 32).

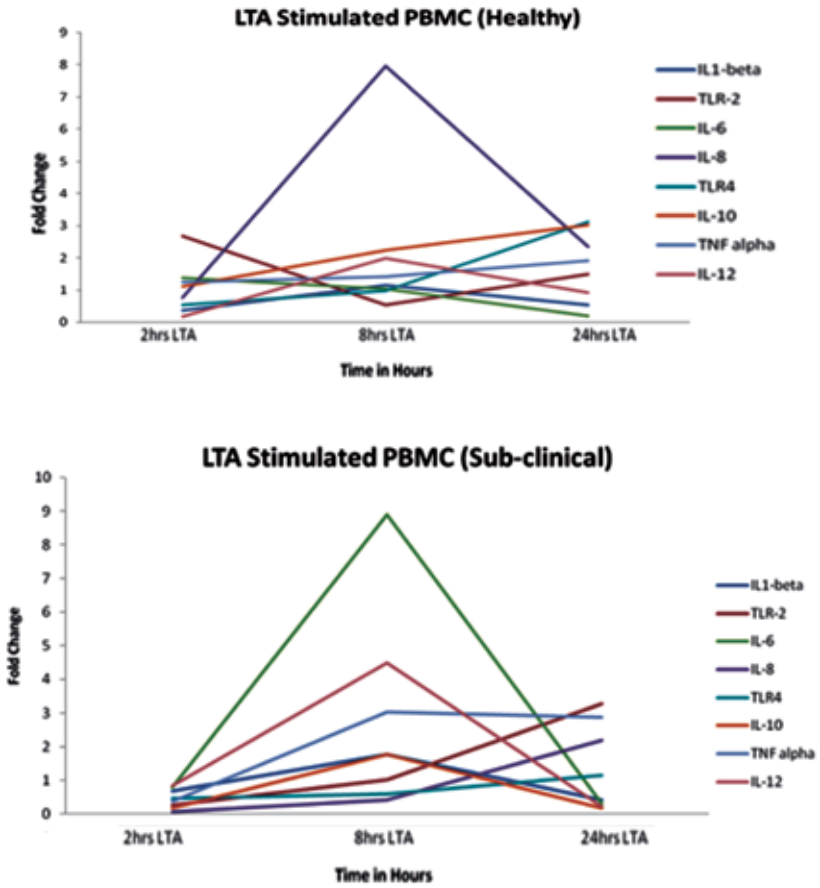


Fig. 32: LTA stimulation of PBMC-Fold change in expression levels observed for selected PRR (TLR2 and TLR 4) and inflammatory mediators (IL6, IL8, IL10, IL12, TNFα and IL1β) in PBMCs in *in-vitro* culture upon LTA antigen stimulation in subclinical mastitis sample group.

IPC: ANSCNIVEDISOL201200400028 Project ID:OXX01505

Development of Recombinant Antigen Based Diagnostics for Surveillance of Peste des Petits Ruminants

V. Balamurugan, M. Nagalingam and D. Hemadri

The expression of PPR Virus (PPRV) haemagglutinin (H) protein in *Escherichia coli* (BL21) envisaged to evaluate the potential use of recombinant protein as a diagnostic antigen in ELISA for detection of PPR antibody for serodiagnosis/ sero-surveillance. The gene coding sequences of the immunogenic region of PPR vaccine virus H was amplified, cloned and expressed in E.coli. Expression of PPRV H protein was induced with 1mM IPTG in recombinant pET33b+ PPRV H bacterial (BL-21-host) clone. Further, the expression level of protein was optimized by subjecting to various parameters (Fig.33), like concentration of IPTG, temperature (22^0 to 37^0C), duration of incubation (0 to 10 hours), etc., and characterized by SDS-PAGE and Western blot using a PPRV specific serum, anti-HisTag conjugate, that confirmed ~ 45kDa PPRV specific recombinant H protein, which expressed as insoluble form. The gene sequence was further cloned in pET 32b vector along with a fusion protein of Thioredoxin and studied for its expression. Results of all these expression studies showed that, the PPRV H protein was expressed as insoluble fraction (inclusion bodies) in the bacterial host. Then Ni-NTA purification method was standardized for purification of the expressed His-Tag truncated PPRV H protein in bacterial system (Fig. 34). Column refolding methods with different concentration of urea were used and optimized in this study to obtain the expressed protein in native soluble form and it is used as coating antigen in the ELISA for its suitability as diagnostic antigen. To confirm the native expression of the protein, the protein was injected into rabbits for raising immune sera as well as to check the immunogenic nature of the expressed protein. The antibodies raised against the recombinant protein as well as PPRV antigen were checked in ELISA using whole virus antigen. The sera raised against recombinant protein, were reacted well in ELISA when using whole virus antigen, which indicated the expressed protein is immunogenic. Further, the characterization and reactivity of the protein in indirect ELISA was assessed using known positive and negative serum samples with respect to PPRV antibodies to optimize the reactivity and checked with whole PPRV antigen based indirect ELISA. Further standardization of PPRV H protein based indirect ELISA for sero-diagnosis/surveillance of PPR in sheep and goats is in progress.

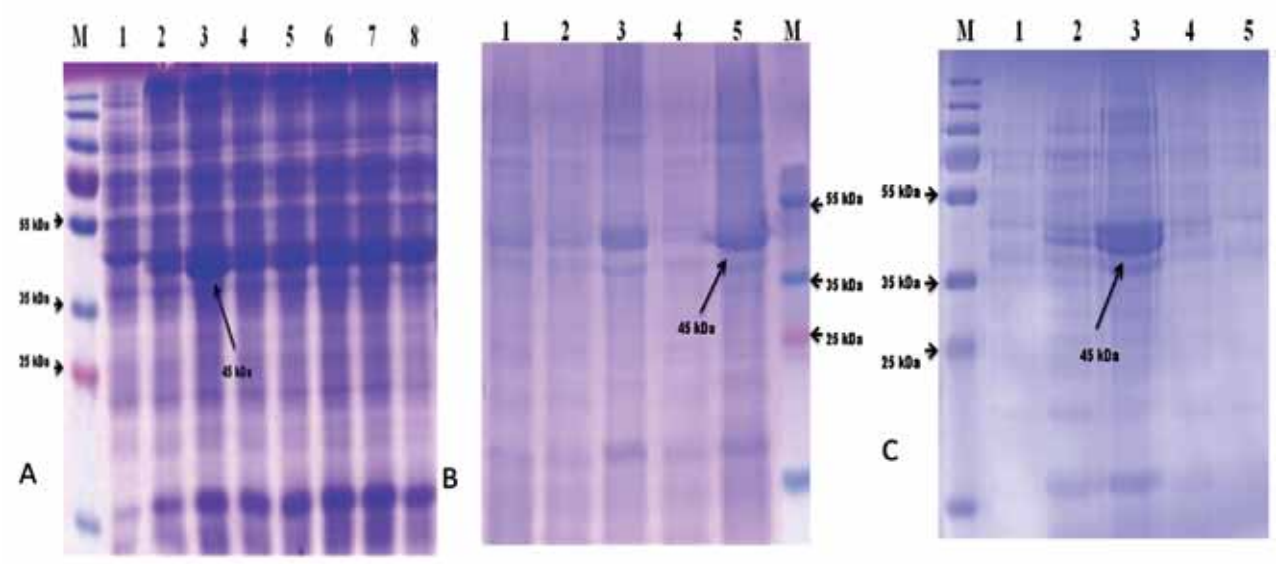


Fig. 33: SDS-PAGE analysis of 33b + HpC.2 C3 showing A. Hour interval: Lane M: Prestained protein ladder plus (Thermo scientific PIERCE) ; Lane 1-8: 100 Mm IPTG Induced hours from 0, 2, 4, 5, 6,7,8,9,10. Optimum -Time-4 Hrs. B. Different levels of Temperature: Lane1-5: Hours 25, 27, 30, 35 & 37. Lane M: Prestained protein ladder plus (Thermo scientific PIERCE marker). Optimum Tempt-37^{0}C. C. different mM Conc. of IPTG: Lane M: Prestained protein ladder plus (Thermo scientific PIERCE marker); Lane1-5: positive clone 33b+ HpC.2 c3 (0 mM conc. 0.5 mM, 1.0 mM, 1.5 mM, 2.0 mM conc.); 1.0 mM / lane 3 showing good level of expression.Optimum Conc.-1 mM IPTG.

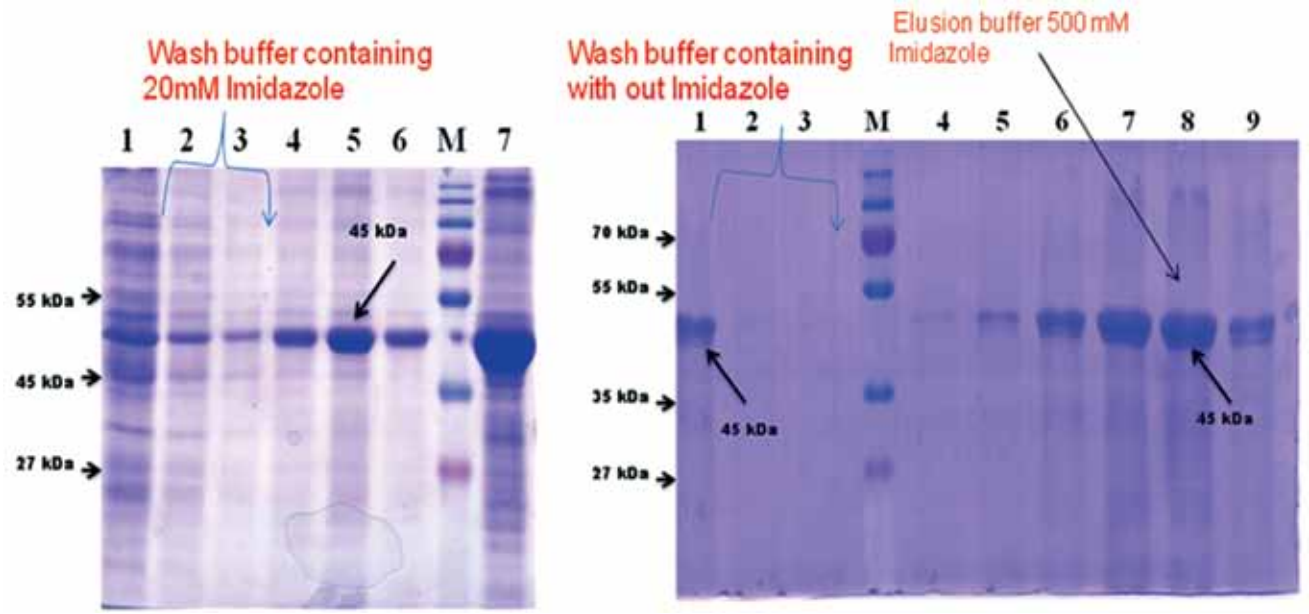


Fig. 34: Purification of pET33b + HpC.2C3 :A. Lane 1-3: Wash 1-3 with imidazole ; Lane 4-6: Elution 1-3 (20mM,100mM, 300mM Imidazole conc.);Lane M- Prestained protein ladder plus (MBI Fermentas, USA passing via., column); Lane 2 to 3: Wash 1 and 3; Lane M: Prestained protein ladder plus (Thermo scientific PIERCE -) Lane 7: 33b + Hp C.2 C3 Crude lysate; B. His- tag purification: Lane1: 33b+Hp2c3 (Before marker; Lane 4-5 : Elution 1-2 with 300mM Imidazole; Lane 6-7: Elution with 400mM Imidazole; Lane 8-9: Elution with 500mM Imidazole.

IPC: ANSCNIVEDICOP2012019 00043 Project ID:OXX02254

Serosurveillance and Association of Toll-like Receptors, Th1-Th2 Status and Viral Genotypes in Susceptibility and Severity of PPR among Goats and Sheep of North East India

V. Balamurugan, M. Nagalingam and D. Hemadri

Seroprevalence and virus genotyping study of PPR virus from sheep and goats in NE India was envisaged to know the status of PPR in North Eastern region.

Serum samples of Goat and Sheep from the North Eastern India submitted collected through AICRP centres of PD_ADMAS and collected sample submitted by the lead Institute to NIVEDI were screened for PPRV-specific antibodies by using PPR Competitive ELISA kit. A total of 391 goat serum samples have been received from 27 districts in the seven states of North Eastern region in India i.e., Meghalaya (Ribhoi, East Khasi Hills, Jaintia Hills districts), Assam (Kamrup, Mongoldoi, Bongaigaon, Nalbari, Udalgiri, Darrang, Gorchuk, Guwahati, Barpeta and Dhubri districts), Manipur (Ukhrul, Thoubal, Imphal East, Senapati, Bishnupur, Churachandpur and Chandel districts), Nagaland (Kohima), Arunachal Pradesh (West Kameng, Lohit, West siang and Papum Pare), Tripura (West Tripura, Bishalgarh) and Mizoram. In addition, 35 sheep serum samples from four districts of Manipur (Churachandpur, Imphal East, Senapati and Ukhrul) were also received.

On analysis of 318 random goats serum samples by PPR c-ELISA, 37 samples were found positive for PPRV specific antibodies, which indicates that an overall seroprevalence of 11.63% in goats. All the serum samples from sheep were found negative for PPRV antibodies. Further, on analysis of the serum samples collected during outbreaks investigation places especially in Assam, showed overall seroprevalence 47.82% (33/69) of PPR. The clinical blood and tissues samples collected during outbreaks were tested for PPRV antigen/nucleic acid by using various serological and molecular assays including isolation study in Vero cells. These test results, confirmed the each outbreaks occurred in various districts of Assam states as PPR.

Further, the serum samples (n=80) from PPRV antibodies positive and negative goats samples were further subjected to serum biochemical parameters using commercially available kit namely Autospan Liquid Gold, (Span Diagnostics Ltd, India) for Total protein Test kit (Biuret method), Glucose (GOD-POD method), Albumin estimation (BCG method), ALT/AST estimation by IFCC/UV-kinetic methods. The test results showing that there is no significant difference in the glucose, total protein, aspartate amino transferase (AST) between the PPRV antibodies positive goats when compared to PPRV antibodies negative animals. However the albumin level showed a non significant increase and globulin level decreased in PPRV antibodies positive goats. The ALT showed a non significant increase in PPRV antibodies positive animals. The globulin level decreased in PPRV antibodies positive animal may be due to lymphopenia caused by the viral infection. The alanine transaminase (ALT) showed slight increase which might be due to liver tissue damage due to PPRV infection or some other bacterial infection affecting the liver in animals.

In conclusion, the presence of PPRV antibodies demonstrate that goats were exposed to PPRV infection and it implies the importance of disease in North Eastern region besides widespread presence of the disease in sheep and goats in rest of endemic India. Further, the study showed that the prevalence of PPRV antibodies in apparently healthy goats under natural situation, approximately 11 % of the animals were protected from re-infection of PPRV. This in turn help in the implementation of disease control strategies such as vaccination in that particular geographical area.

IPC: ANSCNIVEDISOP201200600030 Project ID:OXX01504

All India Network Program on Bluetongue

D. Hemadri

Bluetongue is a vector-borne disease sheep, cattle, goats and other domestic and wild ungulates. The bluetongue outbreak data of 2001-13, from Andhra Pradesh was collected and analyzed. The data, which was obtained from the AH department, was both aggregated at the district level (2001-2008) and also available at the village level (2009-13). In order to have uniform data structure for analysis, the village level data was aggregated to district level. To calculate district level sheep population for various census years was also obtained from AP state AHVS department and used for the analysis purpose. Village geographic coordinates were extracted from internet. Given below are salient findings from the analysis of the data;

Seasonality: Month-wise distribution of bluetongue outbreaks is given in the Fig. 35. From the figure it can be seen that like many other states in India, disease is seasonal in Andhra Pradesh. The season is very long starting from south west monsoon to the end of north east monsoon. Compared to the two south Indian states of Karnataka, Tamil Nadu, the disease season sets in early (from August-September); soon after the monsoon rains.

From the data it can also be said that most outbreaks occur during September-December indicating that period of the year is suitable for growth and replication of the culicoides vector.

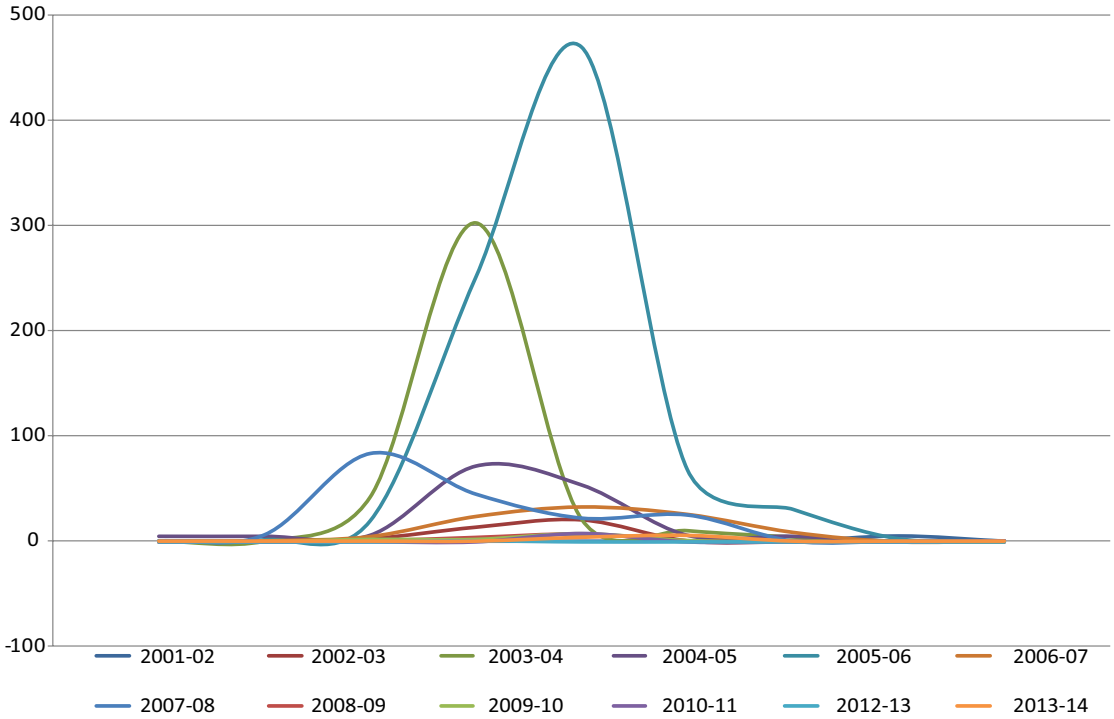


Fig. 35: Month-wise distribution of bluetongue outbreaks in Andhra Pradesh(2001-14)

Outbreaks

A total of 1744 outbreaks of bluetongue occurred in Andhra Pradesh from 2001-2013. During these outbreaks, 298201 cases and 66556 deaths were recorded. Year-wise representation of the outbreaks is shown in the (Fig. 36.), which, indicates cyclical nature of the outbreaks and a decreasing trend in the number of outbreaks.

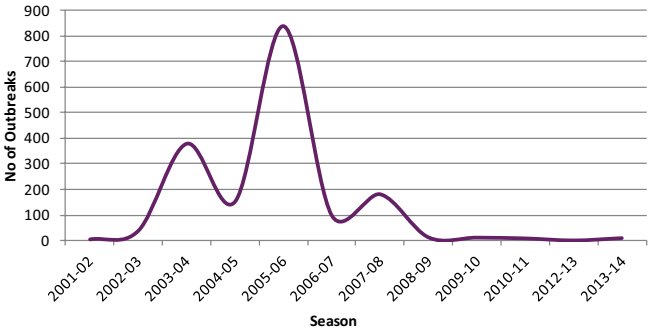


Fig. 36: Year-wise distribution of BT outbreaks in AP

From the data analysis, it was evident that outbreaks were reported from Krishna district in 9 of the 11 seasons. Nellore, Nalagonda, Prakasam and Srikakulam districts accounted for 8 of the 11 seasons. Highest numbers of outbreaks (226 & 225) were recorded from Nellore & Rangareddy districts. Similarly larger numbers of outbreaks were recorded from Mahaboobnagar (184), Prakasam (179), Nalgonda (145) and Guntur (132) districts. Interestingly, although large number of outbreaks have been recorded from Rangareddy and Mahaboobnagar, the disease occurred only for two and three seasons respectively in these districts.

Outbreaks versus Cases

Year-wise distribution of average number of cases per outbreak is given in the Fig. 37. From the figure it can be seen that severe outbreak occurred during the 2005-06 season (average 143 cases/OB), followed by 2003-04 season (average 67 cases/OB). The severity of the outbreaks can be judged by the fact that almost all districts of AP barring Adilabad and West Godavari were affected. Similarly, in 2003-04 season, Kadappa, Kurnool and Nizamabad districts were not affected. Districtwise distribution of average number of cases per outbreak for 2003-04, 2005-06 and 2008-09 season is given in the Fig 38. It is evident that the districts viz., Ananthapur, Kadapa, Mahaboob nagar, Rangareddy, Srikakulam, Warangal accounted for more than 150 cases/ob during 2005-06 season. And obviously these were more severely affected during that season. Similarly, West Godavari, Rangareddy and Nalagonda were more severely affected (more than 130 cases/ob) during 2003-04 season. Interestingly, Rangareddy was the only district to be severely affected both the seasons.

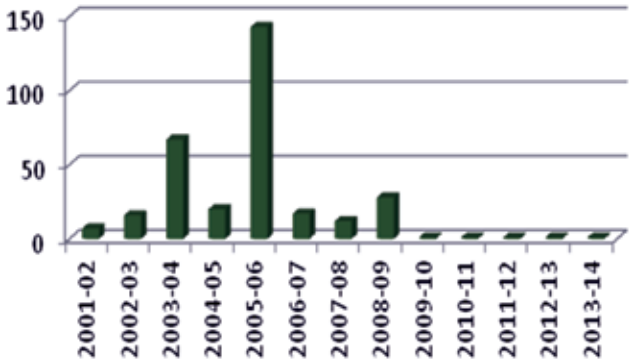


Fig. 37: Average number of cases per outbreak

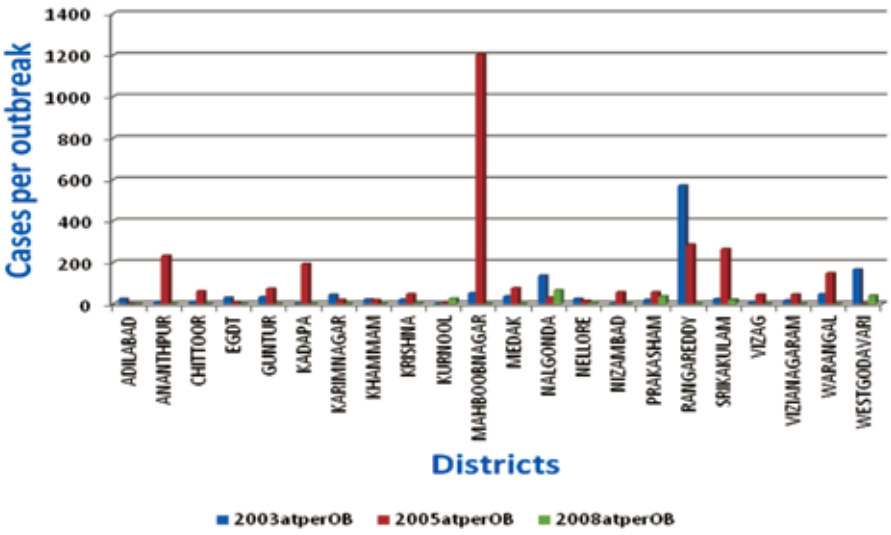
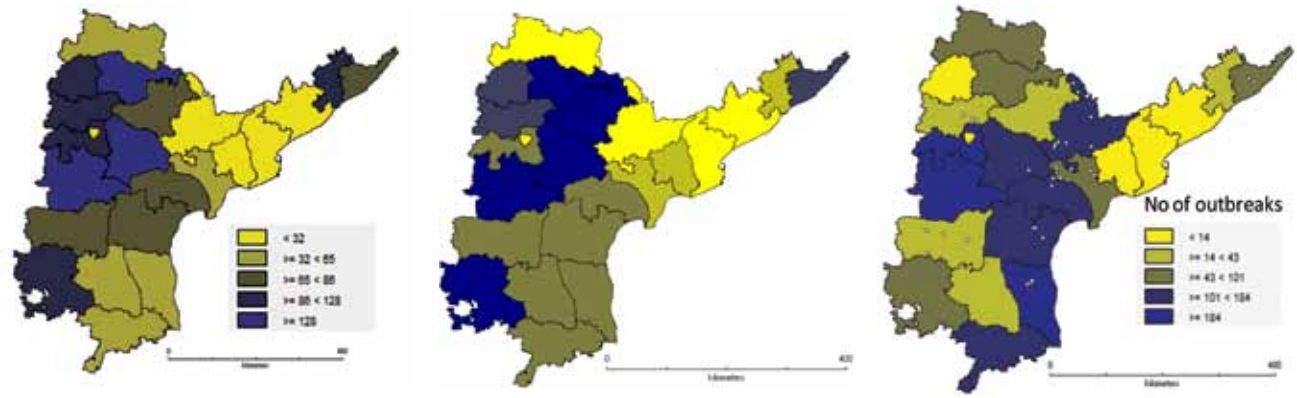


Fig. 38: District-wise distribution of average number of cases per outbreak

Population versus cases

Unlike Karnataka and Tamil Nadu, every district in Andhra Pradesh harbours sizeable sheep population. Census-wise distribution of sheep in AP is given in Fig. 39 which, show an increasing trend in sheep population. The spatial distribution of bluetongue outbreaks is given in the Fig. 40.



2003 2007

Fig. 39: Distribution Sheep (Density/Sq Km) in AP

Fig. 40: The spatial distribution of bluetongue outbreaks

Krishna, Srikakulam, Prakasam & Nellore districts harbor moderate sheep population (65-86 sheep/ sq. km). Curiously, the latter two districts were involved in large number of outbreaks as well as outbreaks were recorded in eight of the 11 seasons. Krishna district has about 50 sheep/sq. km and was accounted for outbreaks in nine of the 11 seasons and 81 outbreaks. It is important to note that outbreaks were not proportional to the sheep population, nevertheless they occurred in almost all the seasons in these districts indicating there some niche contributing to the outbreaks in these districts.

Serotypes : As many as seven serotypes have been isolated from Andhra Pradesh during the period. The details are given below. Filled cells indicate the isolation of that serotype for that year.

Sero-type/ Year	2002-03	2003-04	2005-06	2006-07	2007-08	2010-11	2011-12
BTV-9							
BTV-10							
BTV-21							
BTV-16							
BTV-1							
BTV-2							
BTV-12							

(Data source: VBRI, Hyderabad)

Serological Survey of Bluetongue in Karnataka

Bluetongue is endemic and seasonal in Karnataka and on an average 40-50 outbreaks are reported annually. Curiously, very few outbreaks were recorded during 2011-13. In order to assess the situation a serological survey was undertaken. Results of the survey are given as surface map. The results indicated that most of the sheep in Karnataka (nearly 70% of the population harboured BTV antibodies) (Fig. 41) and the survey indicated several areas in Bagalkot, Chitradurga, Tumkur, Raichur, Koppal, Bijapur and Belgaum districts as high prevalence areas. Interestingly reported outbreak locations matched these high prevalence areas (Fig. 41). Thus

the study indicated that although disease occurred, the disease reporting was poor and reporting occurred only when there is severe problem.

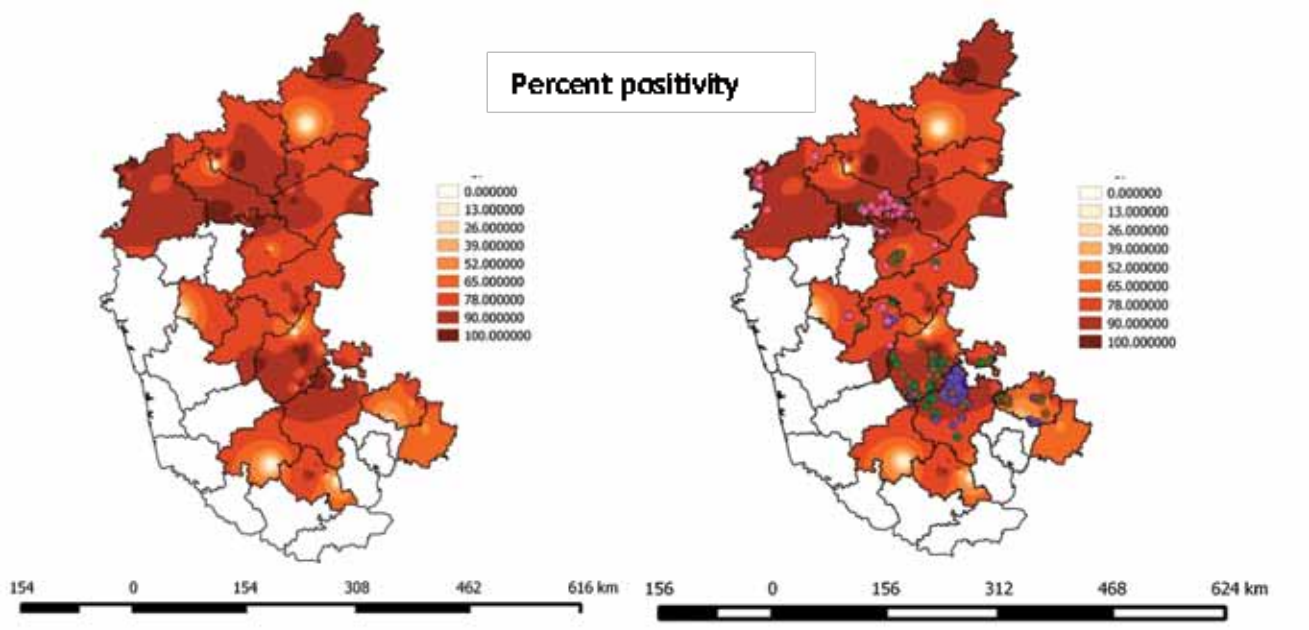


Fig. 41:Surface map of BTV antibodies prevalence. The same with BT outbreak locations.Dots indicate outbreak locations

IPC: ANSCNIVEDISOL201300100044 Project ID:OXX02579

Molecular Diagnosis and Epidemiology of Rabies in Livestock

G.B. Manjunatha Reddy

A total of 36 samples were found to be positive by dFAT and or RT-PCR out of 68 suspected rabies samples different species of animals including wild animals collected from different states of the country. The dFAT was found more sensitive on fresh samples, where as less sensitive on decomposed samples. The RT-PCR was found to be more sensitive on both fresh and decomposed samples. The specific PCR product after purification by commercial kit was subjected for sequencing to private firm and the obtained sequences were edited and phylogenetic analysis was carried out after comparing with already published sequences. The partial N gene sequencing revealed all the isolates belongs to classical rabies virus genotype-I (Fig. 42). There was significant difference in the rabies virus circulating in different species from same region, but it was observed that the isolates formed different cluster/ clades depending on geographical location. One more interesting finding was there was more close relationship between the canine and wild animals rabies virus isolates giving a clue that there might have been species spill over mechanism occurred for which detailed study is warranted in future.

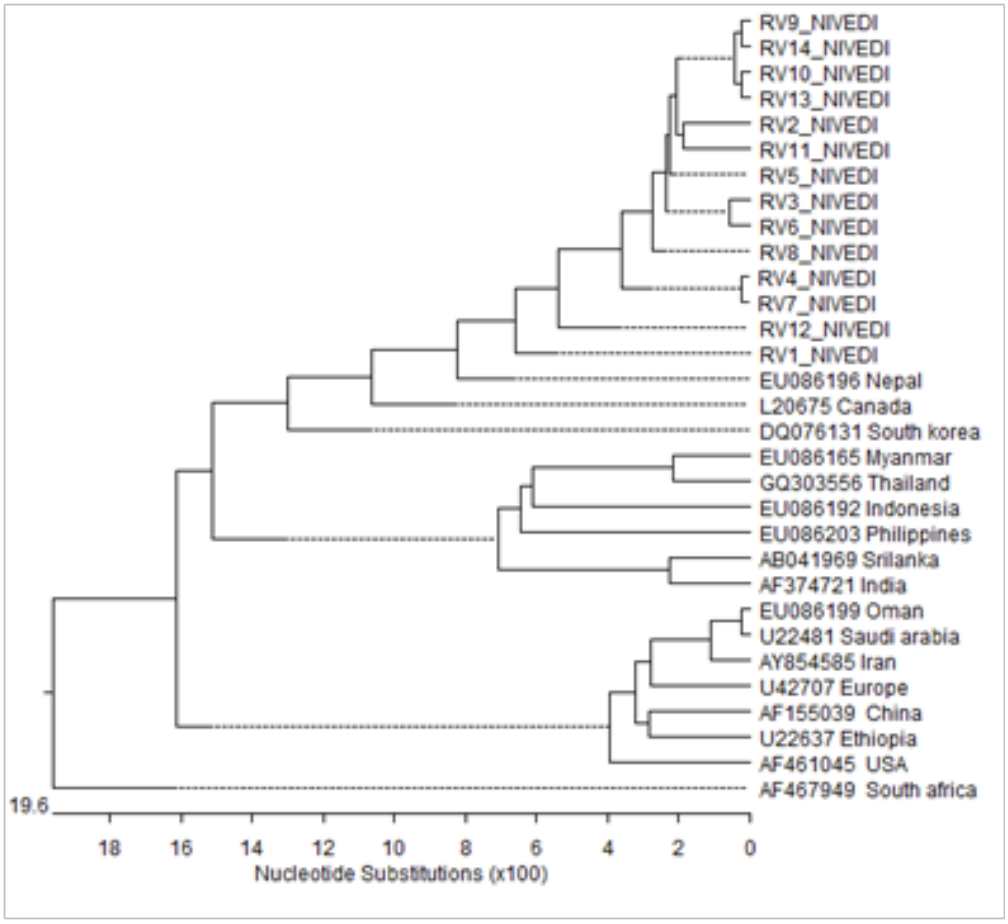


Fig. 42: Phylogenetic tree was constructed (DNASTAR) for N gene sequences from present study and compared with other published sequences revealing all the isolates belonging to genotype-I of rabies virus and are of arctic lineage

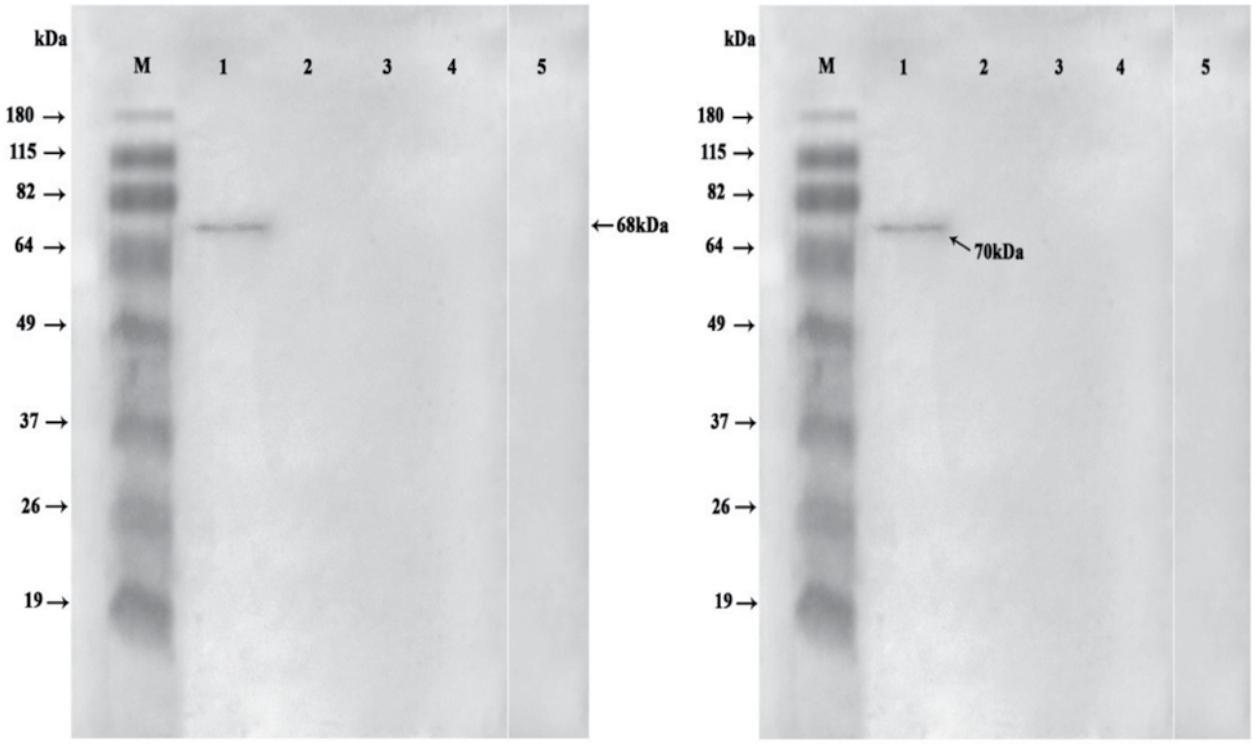
IPC: ANSCNIVEDISOL201200500029 Project ID:OXX01506

Development of Newer Economical, Sensitive Diagnostics for the Detection of Carrier Status of Surra for Surveillance

P. P. Sengupta, V. Balamurugan and M. Nagalingam

The evaluation of recombinant proteins VSG and ISG as diagnostic antigens, were done . The expressed proteins showed immunoreactivity against the hyperimmune sera in both ELISA and Western blot. The expressed proteins were having high level of sensitivity and specificity (more than 90%). The monoclonal antibodies against these proteins were developed and characterized.

The hybridoma cells were produced with fusion of myeloma cells and spleen cells of BALB/C mice immunized against glycoproteins of *T. evansi*. Thereafter, the monoclones were selected by spreading the hybridoma cells in dilution. The monoclones were further selected through immunoreactivity in ELISA and Western blot (Fig. 43). Isotyping analysis the monoclones showed IgG 2b and IgG3 type.



VSG ISG

Fig. 43: The monoclonal antibody against VSG and ISG showed immune reactivity in Immuno-blot assay.

Service Projects

IPC: ANSCNIVEDISIL201100100020 Project ID: IXX08329

National Animal Disease Referral Expert System (NADRES)

M. R. Gajendragad, K.P. Suresh and G.B. Manjunatha Reddy

The National Agricultural Technology Project (NATP) mission mode projects on "Weather based animal disease forecast" (WB_ADF) and "Animal Health Information System through Disease Monitoring and Surveillance (AHIS_DMS)" were launched during the year 2000-01 at PD_ADMAS . The outcome of these projects was National Animal Disease Referral Expert System (NADRES). It is an innovative, web based, dynamic and interactive livestock disease relational database supported by Geographic Information System (GIS) which serves as an epidemiology software. This software addresses the needs of data collection, transmission, retrieval, analysis of critical reporting of disease events as and when they occur and useful for field veterinarians, administrators, technocrats, research personnel, farmers, academician.

The following databases are maintained and up-dated regularly.

- Livestock disease profile
- Demography
- Livestock population profile.
- Meteorological profile
- Land utility and crop production profile
- Agro-ecological profile

Livestock disease profile: The diseases were ranked based on the outbreak reports from 1987 to 2014. FMD and Sheep & Goat pox, HS and BQ and Fascioliasis and Babesiosis is respectively the top viral, bacterial and parasitic diseases recorded (Fig. 44). Similar trend was observed during 2013-14 (Fig.45).

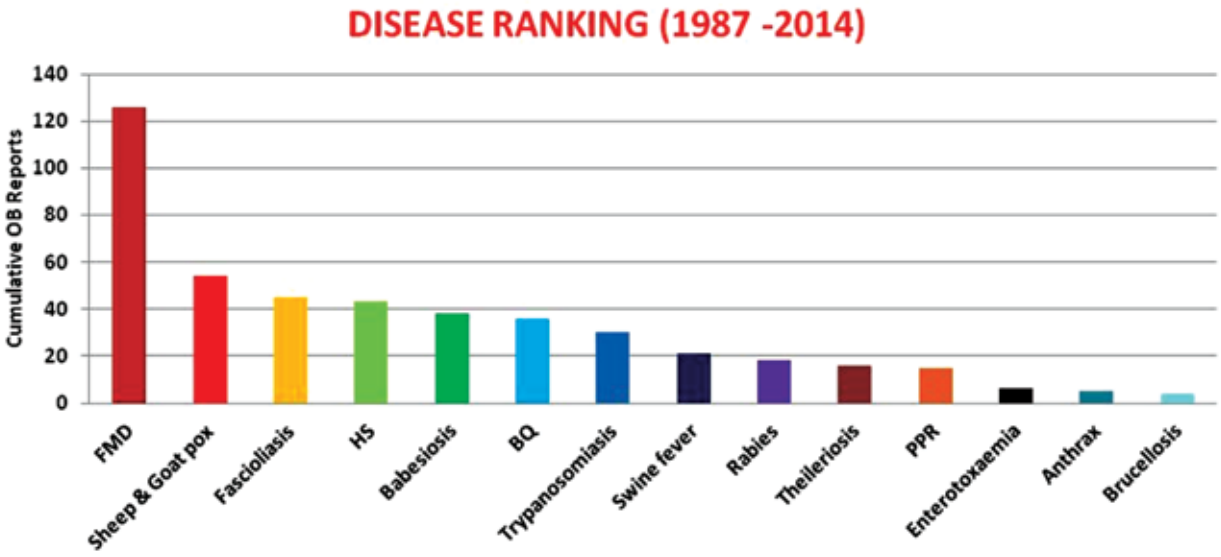


Fig. 44: Disease ranking based on outbreaks in the country

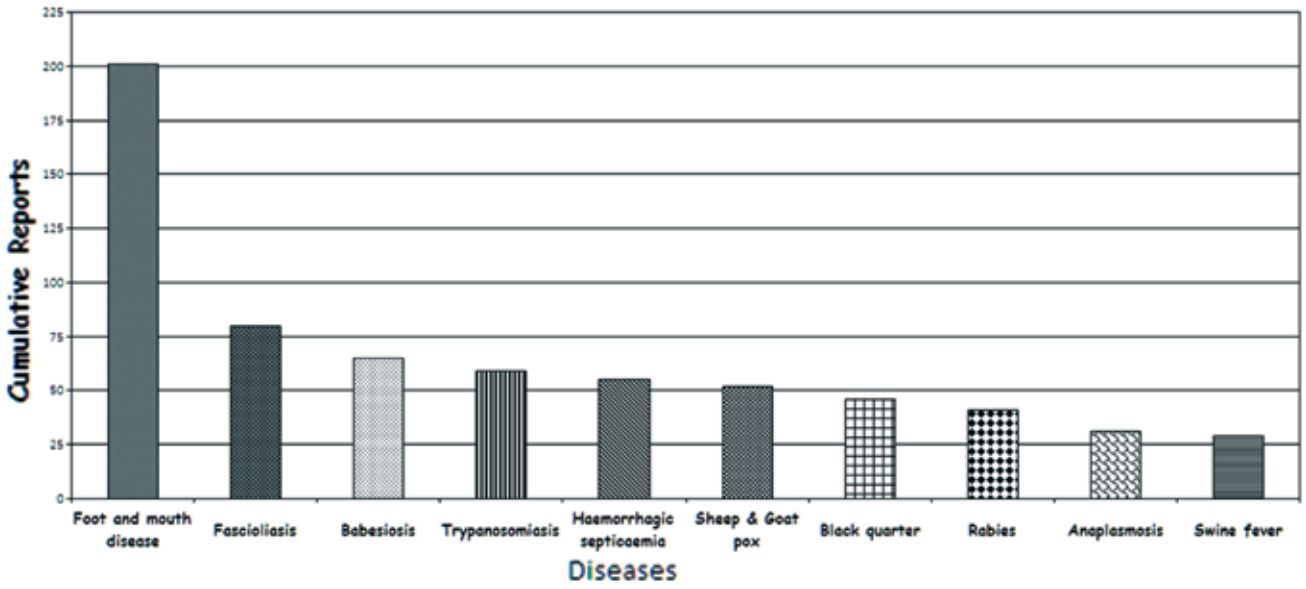


Fig. 45: Top ten diseases based on outbreaks reported during 2013-14

Haemorrhagic Septicaemia: (1987-2014)

Haemorrhagic Septicaemia (HS) continues to be the top bacterial disease being reported in the country over decades. Although the disease trend is declining, it is still alarming in many states. It has been noted that certain districts continue to be endemic from 1987 onwards (Fig. 46). Twenty eight districts have reported the disease for the first time during the period 2011-14 (Table 20).

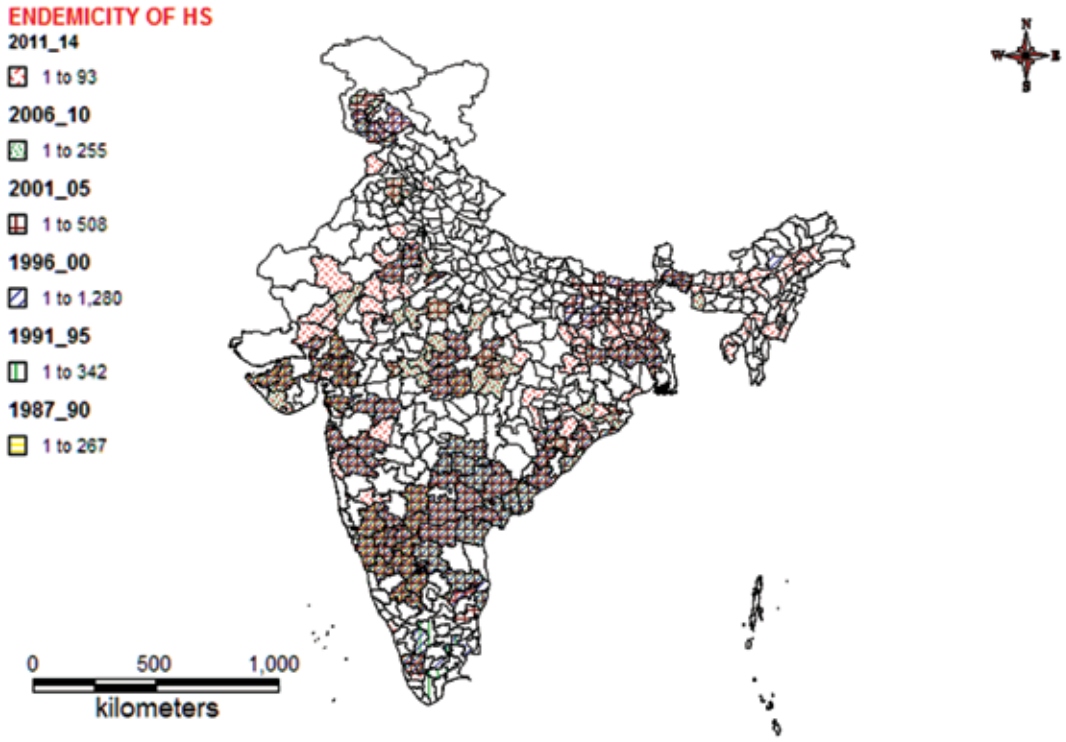


Fig. 46: Endemicity of Haemorrhagic Septicaemia

Table 20: Districts reporting HS for the first time during 2013-14

STATE	DISTRICT
Assam	Barpeta, Karimganj, Udalguri, Baksa, Kamrup Metropolitan
Chandigarh	Chandigarh
Chhattisgarh	Jashpur
Gujarat	Tapi
Jharkhand	Shahdol
Madhya Pradesh	Anuppur, Burhanpur, Gwalior, Serai-kela - Kharsawan
Manipur	Chandel, Temenglong
Meghalaya	East garo hills, Southwest Garo Hills
Orissa	Baleshwar, Baudh, Bhadrak
Puducherry	Puducherry
Punjab	Amritsar
Rajasthan	Dungarpur, Jodhpur, Simdega, Sirohi
Tripura	South Tripura, North Tripura

Being most reported disease, HS is recorded from all the zones of the country however it is well recorded from Southern Zone (SZ) , Eastren Zone (EZ) and Westren Zone (WZ). There is a decreasing trend in the disease occurrence throughout the country (Fig. 47). HS has been recorded throughout the year without any seasonality. The month of August recorded the highest number of the cases suggests the post-monsoon occurrence of the disease. It is thus advisable to undertake vaccination in the month of late June or early July.

FOOT AND MOUTH DISEASE (1987-2014): Foot and Mouth Disease (FMD) the top viral disease in the country due to sudden occurrence of it in all the south Indian states during the last quarter of the year 2013. The disease was extremely severe with adult cattle succumb to the disease.

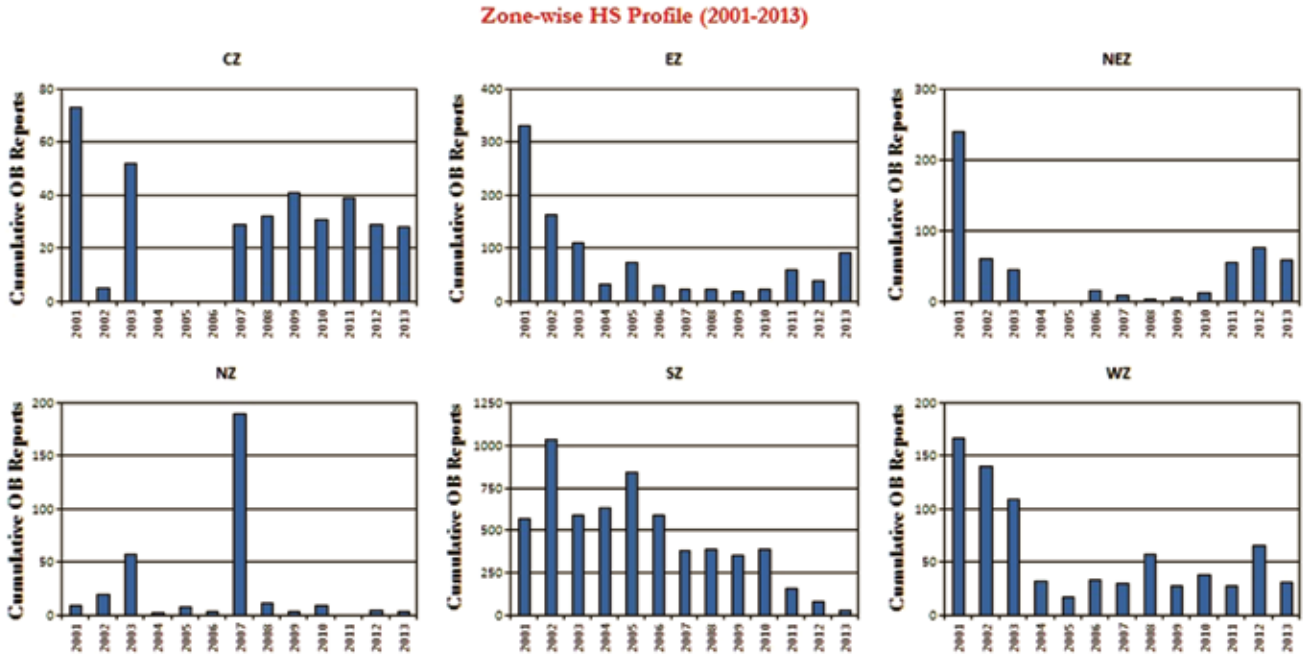


Fig. 47: Zone-wise reports of HS in major states

Endemicity of FMD: It is well known that FMD is endemic in our country but due to sincere efforts of AH departments of the country through national control programme, the disease was waning. However due to a series of outbreaks countered the benefits (Fig. 48). The new districts recording the disease during the period 2011-14 has increased due to these outbreaks (Table 21) .

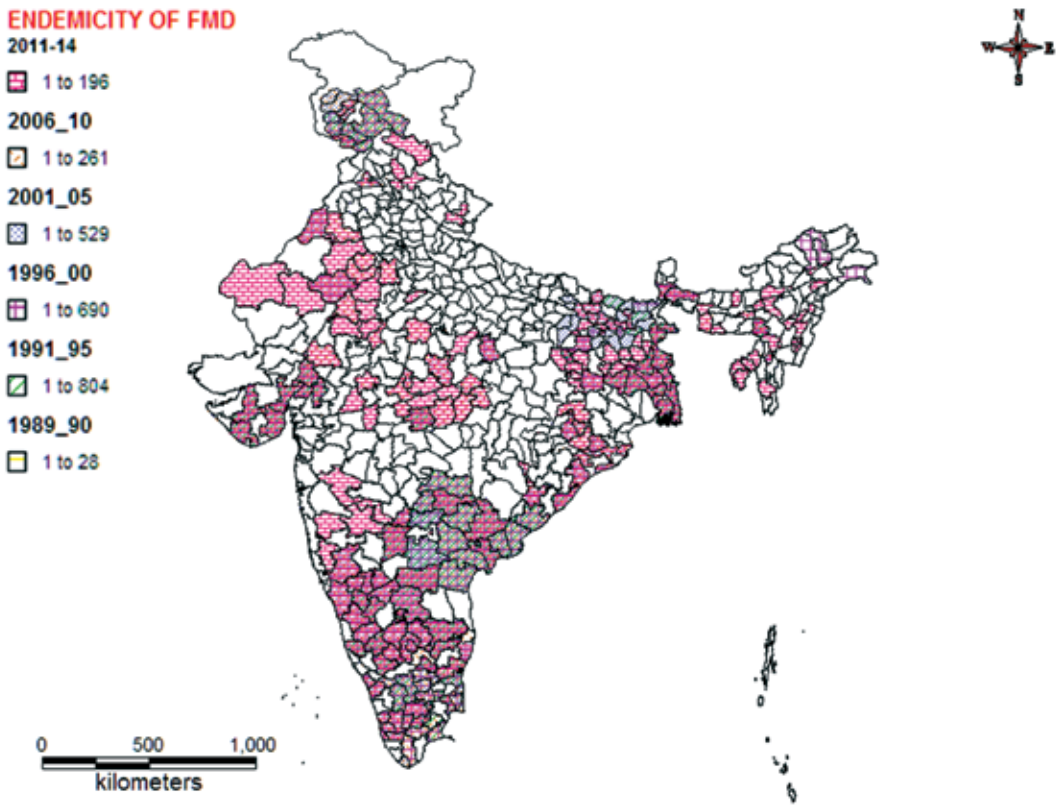


Fig. 48: Endemicity map for FMD

The southern states record FMD with more frequency although in a limited number of outbreak, which indicates that the disease causing pathogen is constantly in the region. A more stringent vaccination regime is required to control the disease in this region. The data from two states, UP and Jharkhand, is not available hence no conclusions can be drawn for them.

Year-wise reporting of FMD: There is a clear cut decline in the number of FMD OBs. This could be due the national control programme being implemented throughout the country. This programme is to be sustained till there is zero reports. Fig. 49 depicts the year-wise FMD trend during the last decade.

Table 21: Districts recording FMD first time during 2013-14

State	District
Bihar	Arwal
Gujarat	Tapi
Jammu and Kashmir	Bandipora, Ganderbal
Jharkhand	Jamtara, Seraikela – Kharsawan, Latehar
Karnataka	Chikkaballapura, Yadgir
Madhya Pradesh	Dewas, Hoshangabad, Narsimhapur, Tikamgarh, West Nimar
Meghalaya	Southwest Garo Hills
Nagaland	Dimapur, Kohima, Peren, Zunheboto
Orissa	Bhadrak
Rajasthan	Bhilwara, Bundi, Churu, Jodhpur, Karauli, Rajsamand, Tonk
Tamil Nadu	Thoothukkudi
Tripura	Dhalai, West Tripura
Uttaranchal	Almora, Bageshwar, Pithoragarh

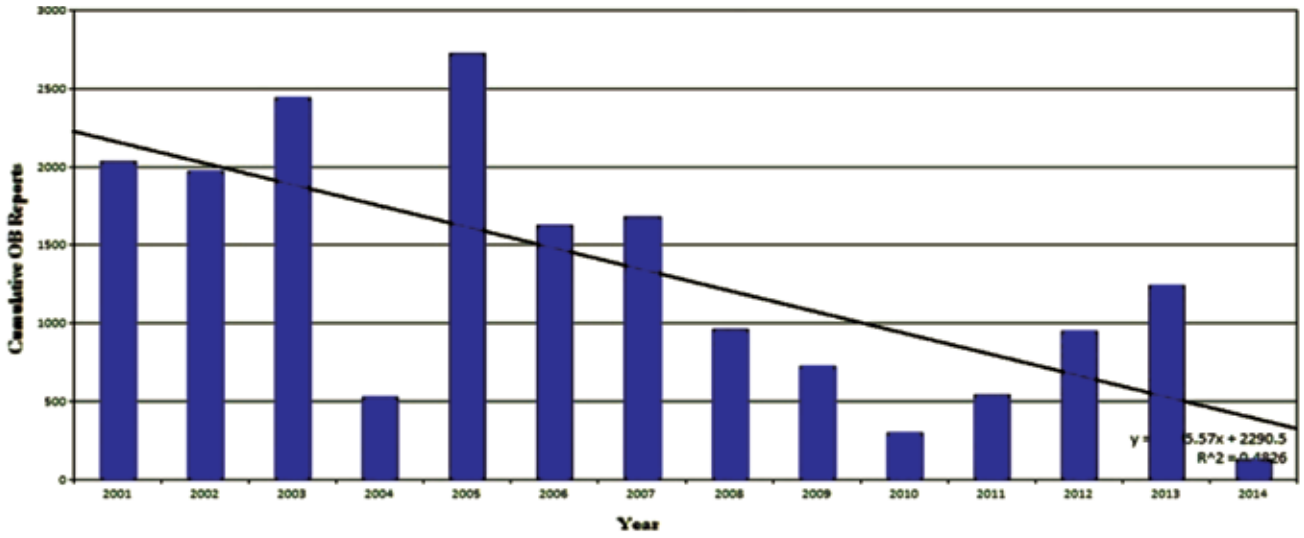


Fig. 49: Year-wise FMD OB reports

All the zones in the country have recorded FMD from 2001 onwards (Fig 50). Though the data is scanty from North Zone (NZ), the disease has been recorded in low intensity throughout the period. South zone has visibly indicated an apparent declining trend but due to sudden increase in the disease outbreaks, the number shot up. East and North East zones (NEZ) show an undulating pattern.

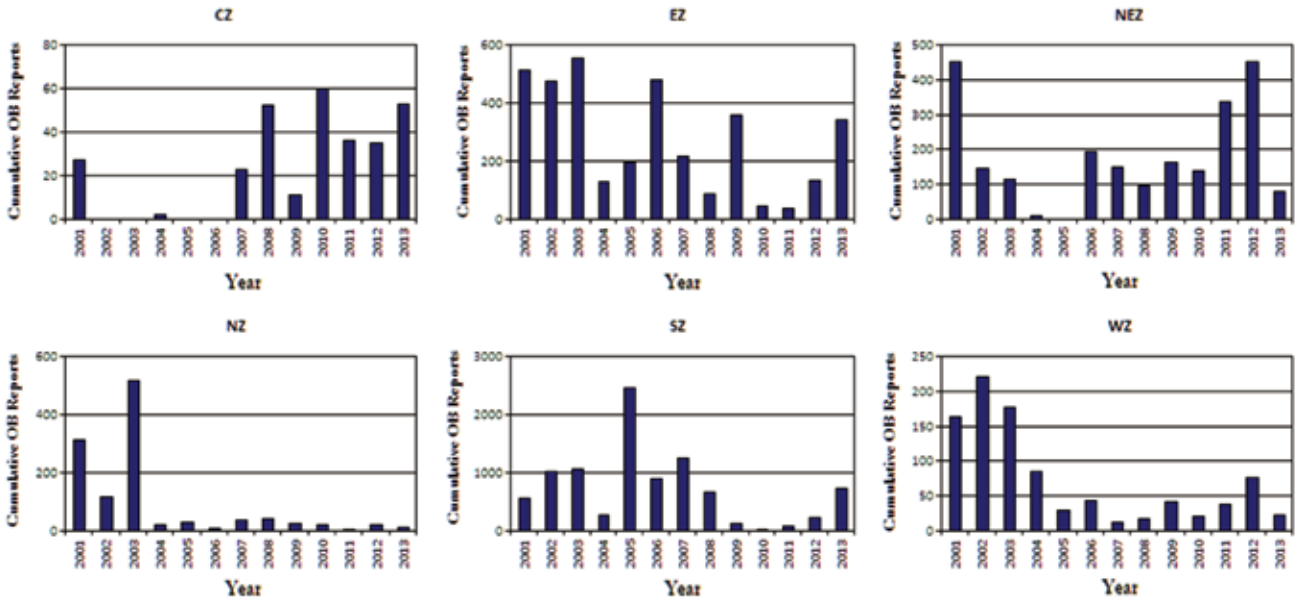


Fig. 50: Zone-wise FMD OB reports (2001-2013)

Fascioliosis : (1990-2014):

There is an increase in reports of parasitic diseases during the period under report. Fascioliosis tops the list. Similar to other infectious diseases, the endemicity of Fascioliosis has been studied. Unlike infectious diseases the data on parasitic diseases is limited except for past two years wherein there is increase in the incidence of Fascioliosis. The incidences are more reported from East zone especially from the state of Jharkhand this could be due to easiness of the diagnosis and limited laboratory facilities needed.

Endemicity of Fascioliosis: The East and North-East zones show high incidence of Fascioliosis with moderate to high severity. The reasoning is difficult since this trend is seen for the past two years. However, it is imperative to note the trend and makes it necessary to implement control measures.

Year-wise reports of Fascioliosis: There is a sudden increase in the incidence of Fascioliosis in the year 2012. This is due to Jharkhand reporting maximum number of cases. This could be inclusion of the fecal sample analysis into the disease data wherein the number of samples are depicted as number of outbreaks. Apart from this, there is a normal distribution of the incidences from 2001 and like any other disease it is showing a declining trend. It is, thus, necessary to understand to use the term "Outbreak" while reporting the disease.

Zone-wise incidence of Fascioliosis: The data on Fascioliasis is limited. The central zone had reported the disease during 2001 only. The East and North-East zones report the disease regularly. This discrepancy could be due to the reason that the disease although diagnosed but never reported. Since parasitic diseases cause heavy economic loss to the farmer and in turn to the nation, remedial steps are necessary for their correct diagnosis, proper reporting and uniform package of practice for their control.

Seasonality of Fascioliosis occurrence: The disease has been reported throughout the year however high incidence has been recorded during winter months (Fig. 51). A systematic study is required for assessing the seasonality vis-à-vis the vector presence.

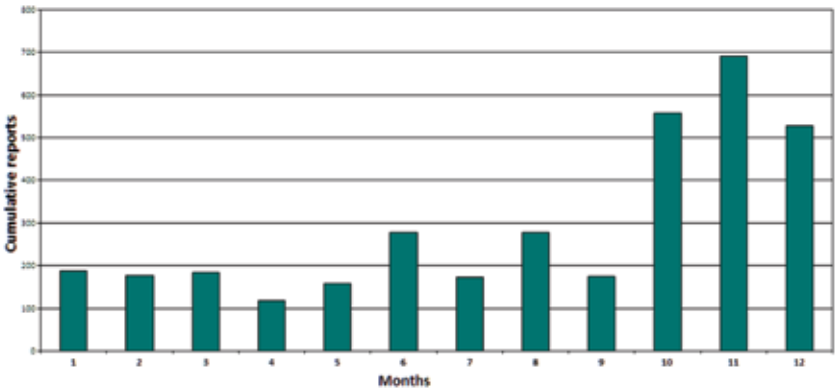


Fig. 51: Month-wise cumulative incidence of Fascioliosis

Demography: For the purpose of epidemiological analysis the country has been divided into 645 districts and each village is considered as a single unit and termed "epi-unit". The new districts added after 2006 have been created in the database. However, the data pertaining to these new districts is being included from 2012 for the purpose of analysis.

Livestock population profile: The latest livestock population data (Livestock census 2007) has been downloaded and formatted. A new database of village level livestock population has been created for the purpose of developing the sampling frame for sero-epidemiology.

Agro-ecological factors: This data has been compiled using the data of the National Bureau of soil survey and land use planning, an ICAR institute. It has been included in the latest database.

Meteorological profile: New set of data is being generated and collated. A new database has been created.

In conclusion, FMD, HS and Fascioliasis continue to dominate the livestock disease scenario of the country. There is a declining trend in the diseases being reported. More number of small ruminants diseases of are being reported. There is an increase in the reports of parasitic diseases. The reporting system of South zone continues to be better than other zones. Reports are being received from certain states which were earlier not submitting them.

IPC: ANSCNIVEDISIL201300200045 Project ID: IXX10708

Seroepidemiology of Bovine Brucellosis

R. Shome, B.R. Shome and M. Nagalingam

Epidemiology of brucellosis in livestock during 2013-14

A total of 9195 random serum samples {cattle (4420), buffalo (1269), sheep (806), goat (2241) and swine (439)} received from 14 AICRP centers were screened for brucellosis. Among 5 livestock species screened, highest seropositivity was recorded in sheep (11.04 %), followed by swine (5.01%); goats (3.93%) and lowest prevalence in cattle samples (3.48%). The state-wise disease prevalence study revealed that highest prevalence was recorded in Punjab {80/419 (19.09%)}; followed by Meghalaya {44/508(8.66%)}, Manipur {57/ 800 (7.13)}. Many states have < 5% seroprevalence like Assam, Kerala, Andhra Pradesh, Jammu and Kashmir, Orissa, Karnataka, Gujarat, M.P., Rajasthan and West Bengal. The brucellosis prevalence in random samples appears to be lower this year than the other two previous years except in sheep (Fig. 52). Also the overall prevalence was found comparatively lower (4.98%) than the purposive samples or samples collected from organized farms (Table 22).

Table 22: Seroprevalence of brucellosis in livestock species during the year 2013-2014

Sl. No.	State	Cattle*	Buffalo*	Sheep**	Goat**	Swine***	TOTAL	Percent Positivity
1	Andhra Pradesh	311 (8)	214 (0)	240 (19)	228 (20)	0	993 (47)	4.73
2	Assam	126 (1)	-	13 (0)	87 (0)	52 (0)	278 (1)	0.35
3	Gujarat	242 (6)	215 (5)	145 (11)	250 (2)	0	852 (24)	2.81
4	Jammu and Kashmir	991 (5)	17	-	-	-	1008(5)	0.49
5	Karnataka	67 (0)	30 (0)	29 (1)	25 (0)	0	156 (1)	0.64
6	Kerala	273 (0)	13 (0)	-	97 (0)	30 (1)	413 (1)	0.24
7	Madhya Pradesh	395(24)	271(1)	0	685 (38)	103(7)	1454 (70)	4.8
8	Maharashtra	395(24)	271(1)	0	685 (38)	103(7)	1454 (70)	4.8
9	Manipur	251 (25)	105 (4)	65 (3)	172 (11)	207(14)	800 (57)	7.13
10	Meghalaya	447 (44)	-	-	19 (0)	42 (0)	508 (44)	8.66
11	Orissa	678 (13)	46 (1)	123 (2)	329 (1)	0	1176 (17)	1.44
12	Punjab	169 (23)	145 (7)	57 (48)	48 (2)	-	419 (80)	19.09
13	Rajasthan	186(0)	103(0)	75(5)	150(2)	0	514(7)	1.36
14	West Bengal	61 (0)	33 (0)	49 (0)	61 (0)	-	204 (0)	0
	TOTAL	154/4420 3.48%	28/1269 2.20%	89/806 11.04 %	88/2241 3.93%	22/439 5.01%	381/9195 4.14%	

*Protein-G ELISA kit; **Sheep & Goat iELISA kit, *** Laboratory standardized swine ELISA protocol

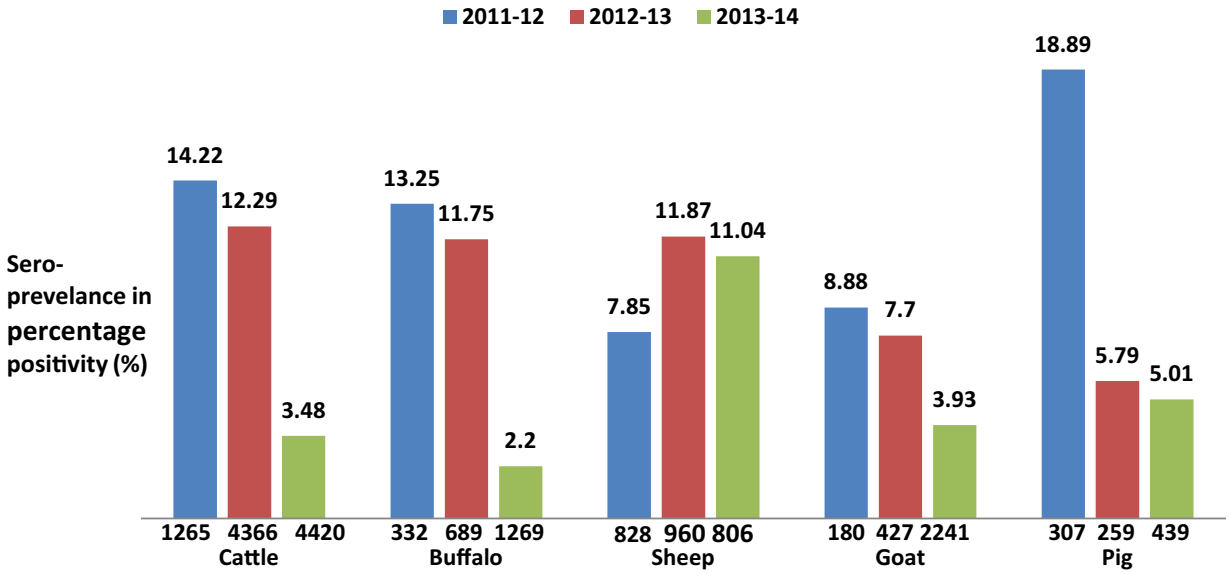


Fig. 52 Yearly trend of brucellosis in different species.

b) Evaluation of Protein – G ELISA kit

During the current year, ICAR-NDDB-IIL Joint Working Group for evaluation ELISA kits have evaluated two indigenously developed kits (Protein G ELISA kit by NIVEDI and other by IIL, Hyderabad). For evaluation, 860 serum samples were sourced from various institutions, coded at PD-FMD, Mukteswar and tested by these kits at their respective institutions. The statistical analysis showed 88.79% and 97.74 % diagnostic sensitivity and specificity with kappa value of 0.85 (kappa value of 0.8 to 1.0) which is considered as very good score for newly developed diagnostic tests).

IPC: ANSCNIVEDISIL201200700031 Project ID: IXX10496

Seroprevalence of Leptospirosis in Livestock Species

V. Balamurugan, M. Nagalingam, R. Sridevi and D. Hemadri

Leptospirosis is the most widespread and fastest re-emerging anthropozoonosis. The disease affects a variety of animals resulting in heavy economic losses to the farming community on account of abortions, stillbirths, infertility and reduced productivity. *Leptospira* reference antigen panels are regularly screened and sub cultured every week in EMJH media for preparation of antigen for MAT. Reference strains of different serovars representing the serogroups in EMJH semi solid media were maintained and used in the study.

Study area 1: Random non-purposive serum samples collected (n= 553) from Coastal districts of the five states (states namely Kerala, Maharashtra, Gujarat, Tamil Nadu and Andhra Pradesh). Study area 2: Purposive random serum samples (n=300) from Navarai, Gujarat. Study area 3: West Bengal: Random non-purposive serum samples (n=42). Study area 4: Odisha: Random non-purposive serum samples (n=414).

All the serum samples were subjected to microscopic agglutination test (MAT) by employing the references serovars as shown in Table 23.The serovars were selected cause disease in livestock or may be of use as sentinel serovars to measure the potential spread.

Table 23: Details of the *Leptospira* reference serovars used in the MAT

Species	Serovar	Strain	Serogroup
L. interrogans	Australis	Ballico	Australis
L. interrogans	Bankinang	Bankinang 1	Au¬tumnalis
L. interrogans	Canicola	Hond Utrech IV	Canicola
L. interrogans	Hardjo	Hardjoprajitno	Sejroe
L. interrogans	Hebdomadis	Hebdomadis	Hebdomadis
L. interrogans	Pyrogenes	Salinem	Pyrogenes
L. borgpetersenii	Tarassovi	Perepelicin	Tarassovi
L. interrogans	Icterohaemorrhagiae	RGA(ATCC443642)	Icterohaemorrhagiae
L. interrogans	Pomona	Pomona	Pomona
L. Santarosai	Shermani	1342 K	Shermani
L. inadai	Kaup	LT 64 - 68	Tarassovi
L. kirschneri	Grippotyphosa	MoskvaV	Grippotyphosa
L. fainei	Hurstbridge	BUT 6	Hurstbridge
L. borgpetersenii	Javanica	Poi	Javanica
L. noguchii	Panama	CZ 214 K	Panama
L. interrogan	Djasiman	Djasiman	Djasiman
L. interrogan	Copenhageni	M 20	Icterohaemorrhagiae
L. interrogan	Bataviae	Swart	Bataviae
L. biflexia	Patoc	Patoc 1	Semaranga

Seroprevalence of leptospirosis in different livestock species

The overall seroprevalence of 24.96% (138/553) with 20.48% in cattle, 20% in buffaloes, 58.62% in goats and 38.32% in sheep was observed while testing coastral areas samples from five states. In Gujarat, the overall seroprevalence of 23% (69/300) with 10.14% in cattle, 10.14% in buffaloes, 79.71% in goats was observed while testing the purposive samples using 15 serovars. In West Bengal, the overall seroprevelance was 80.95% (34/42) with 60% (6/10) in cattle, 100% (10/10) in goats, with 81.8% (9/11) and 81.8% (9/11) in sheep when tested in MAT using 19 reference leptospiral serovars.

In Odisha, the overall seroprevalence of 42.5% with 48.5% in cattle, 28.1 % in bullocks and 45.0 % in bulls was observed when tested non-purposive samples collected during April-May 2013 after cyclone period using 14 serovars. This study supports that bovines may have a role in maintaining Australis serovar apart from being a well known reservoir for Hardjo serovar in Odisha state, India. The prevalent serovars of *Leptospira* in livestock species in different costal districts of endemic states are presented in Table 24.

In conclusion, the coastal region of these states or zone is endemic for leptospirosis as indicated by the high seroprevalence on screening for MAT especially eastern part of country viz., West Bengal and Odisha states. The prevalence of *Leptospira* spp. in apparently healthy animals indicates the presence of this agent in the environment, which may be a source of human infection. Knowledge of the serovars is important for understanding the epidemiology of leptospirosis and establishing public health policies aimed at its control. Knowledge of prevalence of serovars in particular geographical area will help in selection of serovars for providing prompt diagnosis and control measures.

Table 24: Seroprevalence of leptospirosis in livestock species in costal districts of endemic states (2013-2014)

State	Type of random samples / survey	No. of Positive samples / No. of samples screened (% Prevalence)	Serovar Prevalence in the region	Serogroup Prevalence in the region
Andhra Pradesh	Non-Purposive samples	16/50 (32%)	Kaup, Shermani, Pomona, Icterohaemorrhagiae, Bankinang / Hardjo	Tarassovi, Shermani, Pomona, Icterohaemorrhagiae, Autumnalis/ Sejroe
Gujarat	Non-Purposive samples	48/212 (23%)	Hardjo, Kaup/Pomona, Hebdomadis, Grippotyphosa, Pyrogenes/Australis/ Tarassovi/Shermani/ Hurstbridge/ Javanica	Sejroe, Tarassovi / Pomona, Hebdomadis, Grippotyphosa, Pyrogenes/Australis/ Tarassovi/Shermani/ Hurstbridge/ Javanica
Gujarat	Purposive samples	69/300 (23%)	Pyrogenes, Tarassovi, Shermani, Icterohaemorrhagiae, Bankinang, Australis, Kaup	Pyrogenes, Tarassovi, Shermani, Icterohaemorrhagiae, Autumnalis, Australis, Tarassovi
Kerala	Non-Purposive samples	52/165 (32%)	Hardjo, Pomona, Bankinang, Icterohaemorrhagiae, Kaup, Javanica, Hurst-bridge, Hebdomadis, Shermani	Sejroe, Pomona, Autumnalis, Icterohaemorrhagiae, Tarassovi, Javanica, Hurstbridge, Hebdomadis, Shermani
Maharashtra	Non-Purposive samples	19/53 (35%)	Hurstbridge, Hardjo, Kaup, Javanica, Grippotyphosa, Australis/Shermani/ Tarassovi	Hurstbridge, Sejroe, Tarassovi, Javanica, Grippotyphosa, Australis/ Shermani/Tarassovi
Odisha	Non-Purposive samples (April-May 2013)	51/120 (42.5%)	Australis, Hardjo, Kaup, Pyrogenes / Hebdomadis, Javanica / Bankinang	Australis, Sejroe, Tarassovi, Pyrogenes / Hebdomadis, Javanica / Autumnalis
Odisha	Non-Purposive samples (Oct-Dec 2013)	108/294 (36.73%)	Hardjo, Tarassovi, Kaup, Pyrogenes, Pomona, Australis, Hebdomadis, Bankinang	Sejroe, Tarassovi, Bataviae, Hebdomadis, Australis, Pomana, Autmnalis,
Tamil Nadu	Non-Purposive samples	3/72 (4%)	Hardjo / Pyrogenes / Bankinang / Canicola	Hardjo / Pyrogenes / Autumnalis/ Canicola
West Bengal	Non-Purposive samples	34/42(80.95%)	Australis, Bankinang, Icterohaemorrhage, Pomona, Javanica, Pyrogenes, Hardjo , Tarassovi,	Australis, Autumnalis, Icterohaemorrhage, Pomona, Javanica, Pyrogenes, Sejroe, Tarassovi,

IPC: ANSCNIVEDISIL201200800032 Project ID: IXX10709

Seroepidemiology of Infectious Bovine Rhinotracheitis in India

S.S. Patil and D.Hemadri

A total of 6327 bovine serum samples (random sampling) from 15 states during the period under report were screened for the presence of antibodies against IBR using NIVEDI Avidin Biotin ELISA kit and the percent positivity was 52% (Table 25). The cumulative percent positivity of IBR antibodies during 1995-2014 was 36.52% (Table 26). This indicates that IBR antibodies are circulating in the bovine population either due to recent introduction or through sale of infected animals to the neighbouring villages. Cumulative percent positivity during 1995-2014 in Indian bovine population was 36.52% which is still causing a threat to the dairy industry in the form of reproductive issues of the dairy animals.

Table 25: The state wise seroprevalence of IBR in India during 2013-14

Sl. No.	State	No. Tested	No. Positive	No. Negative	Positive Percentage
1	Andhra Pradesh	1069	655	414	61.2
2	Assam	136	56	80	41.1
3	Chhattisgarh	100	66	34	66.0
4	Gujarat	458	143	315	31.2
5	Jammu & Kashmir	676	285	391	42.1
6	Karnataka	236	131	105	55.5
7	Kerala	501	287	214	57.2
8	Madhya Pradesh	650	540	110	83.0
9	Maharastra	331	48	283	14.5
10	Manipur	175	115	60	65.7
11	Meghalaya	351	140	211	39.8
12	Odisha	583	333	250	57.1
13	Punjab	729	367	362	50.3
14	Rajasthan	311	125	186	40.1
15	West Bengal	21	05	16	23.8
	TOTAL	**6327**	**3296**	**3031**	**52.0**

Table 26: The Cumulative seroprevalence of IBR in India during 1995-2014

Sl. No.	Year	No. Tested	No. Positive
1	1995-96	3428	1303
2	1996-97	3521	1096
3	1997-98	1442	599
4	1998-99	1675	767
5	1999-01	6883	2776
6	2001-02	3373	785
7	2002-03	7933	3271
8	2003-04	1300	668
9	2004-06	9564	3507
10	2006-07	2820	1197
11	2007-08	4270	1242
12	2008-09	4821	1423
13	2009-10	4496	1494
14	2010-11	1483	621
15	2011-12	2275	507
16	2012-13	5632	1468
17	2013-14	6327	3296
TOTAL		**71243**	**26020 (36.52%)**

IPC: ANSCNIVEDISIL201100300022 Project ID: IXX08279

Maintenance and Updating of Livestock Serum Repository

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

During the period under report national sampling frame providing village wise account of livestock species (approximately 643000 villages all over India) was developed. Similarly modification to the some of the existing forms (viz., receipt entry form, results entry form, and result communication form) was made as a part of updating the software. Screen shots of access database for reference are given below (Fig. 53). Information for 87 fields in receipt, despatch, results, result communication, storage forms for all the 8813 serum samples were entered.

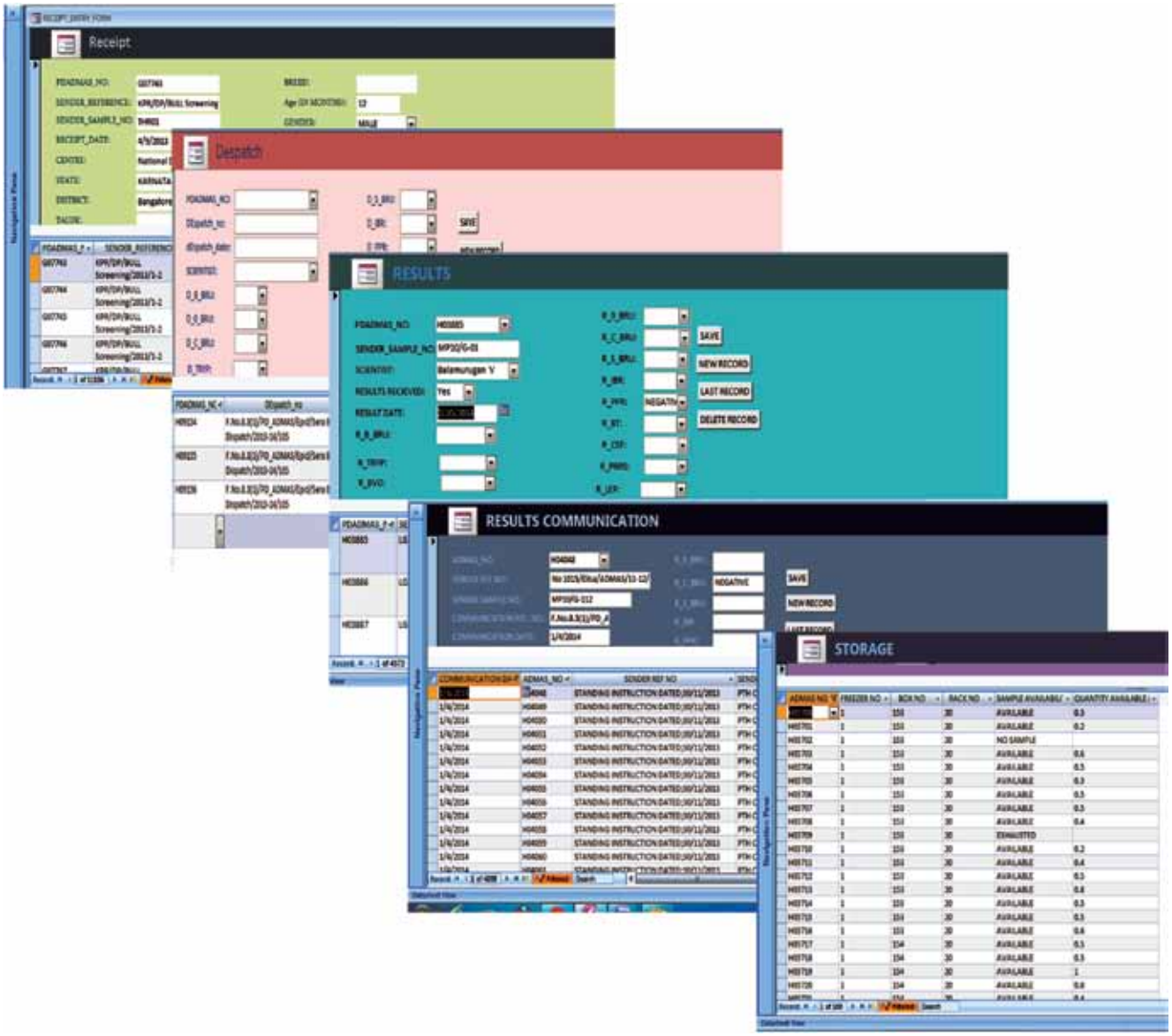


Fig. 53: Screen shots of serum bank software

During the year, 2013-14, 12548 serum samples were received from 18 state/union territories. Given below (Fig. 54) is the distribution of serum samples received at serum repository.

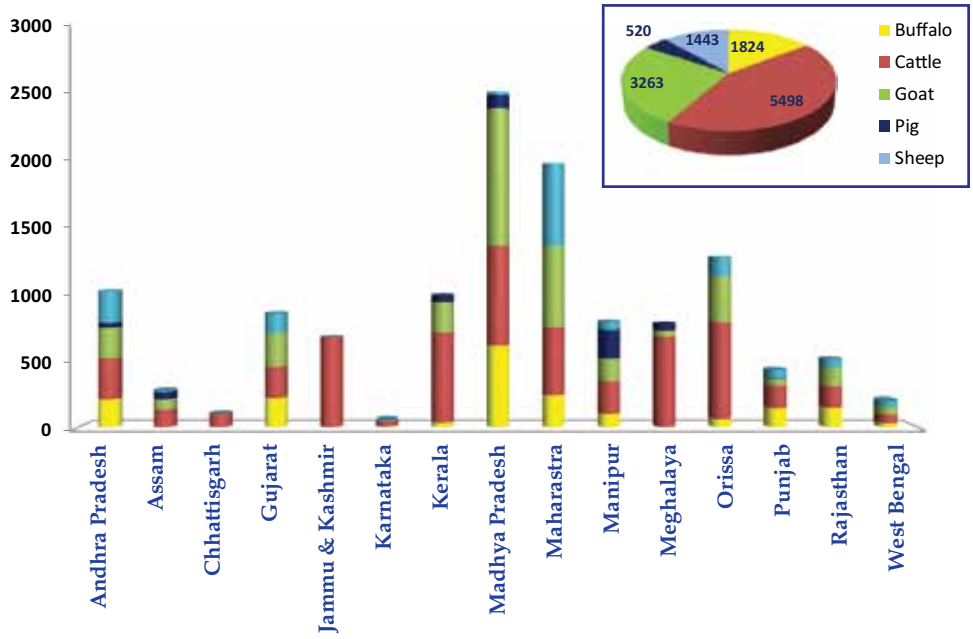


Fig. 54. Distribution of serum samples received at serum repository 2013-14.

Of the 12548 serum samples, 1824, 5498, 3263, 520, 1443 were from buffalo, cattle, goat, pig and sheep respectively. The serum bank arranged the screening of all the samples for antibodies against various pathogens [(*Brucella abortus,* n=7322) (*Brucella melitensis*, n=4706) (*Brucella ovis*, n=300) (*Brucella suis*, n=518), (*BoHV-1*, n=7322), (*CSFV*, n=518), (*T. evansi*, n=933), (*PRRSV,* n=145), (BTV, n=), (*PPRV*, n=176)]

Brucella (*B. abortus*) screening results of the 5285 bovine serum samples indicated that only 182 (3.44%) are positive. Of the 15 states screened, Manipur (16.48%), Punjab (9.58%) and Meghalaya (8.72%) showed higher positivity compared to all other states. Contrary to *Brucella*, another pathogen involved abortions in bovines; BoHV-1 appeared to be more prevalent in the country. Of the 5152 samples about 47.59% of the samples were positive for anti-BoHV-1 antibodies. About 83% (537/642) of the serum samples from MP were positive, indicating that the disease is highly prevalent there. Other states with higher prevalence were Manipur, Orissa and Kerala. Of the 5057 serum samples screened for both bovine brucellosis and IBR, 95 were antibody-positive for both the diseases while 2614 samples were negative for both the diseases. Importantly, 2357 IBR positive samples were negative for brucellosis, while, 86 IBR negative samples were *Brucella* positive.

Similarly, 520 samples were only screened for CSF, while 275 samples of these were screened for both CSF and swine brucellosis. Screening results from pig serum samples from Kerala, Madhya Pradesh, Assam, Meghalaya, Manipur indicated that 7.9% & 41.89% of these were positive for *Brucella* suis & CSFV infection respectively. Of 275, 141 samples were both negative for CSF and swine brucellosis, while, 112 *Brucella* negative samples were CSF positive, while 15 *Brucella* positive samples were CSF negative. 7 samples were both CSF and *Brucella* positive. 11.73% (84/716) of sheep & 4.46% (90/2014) of goat serum samples were positive for *B. melitensis*. Results of the screening for other pathogens are given in the figure 55.

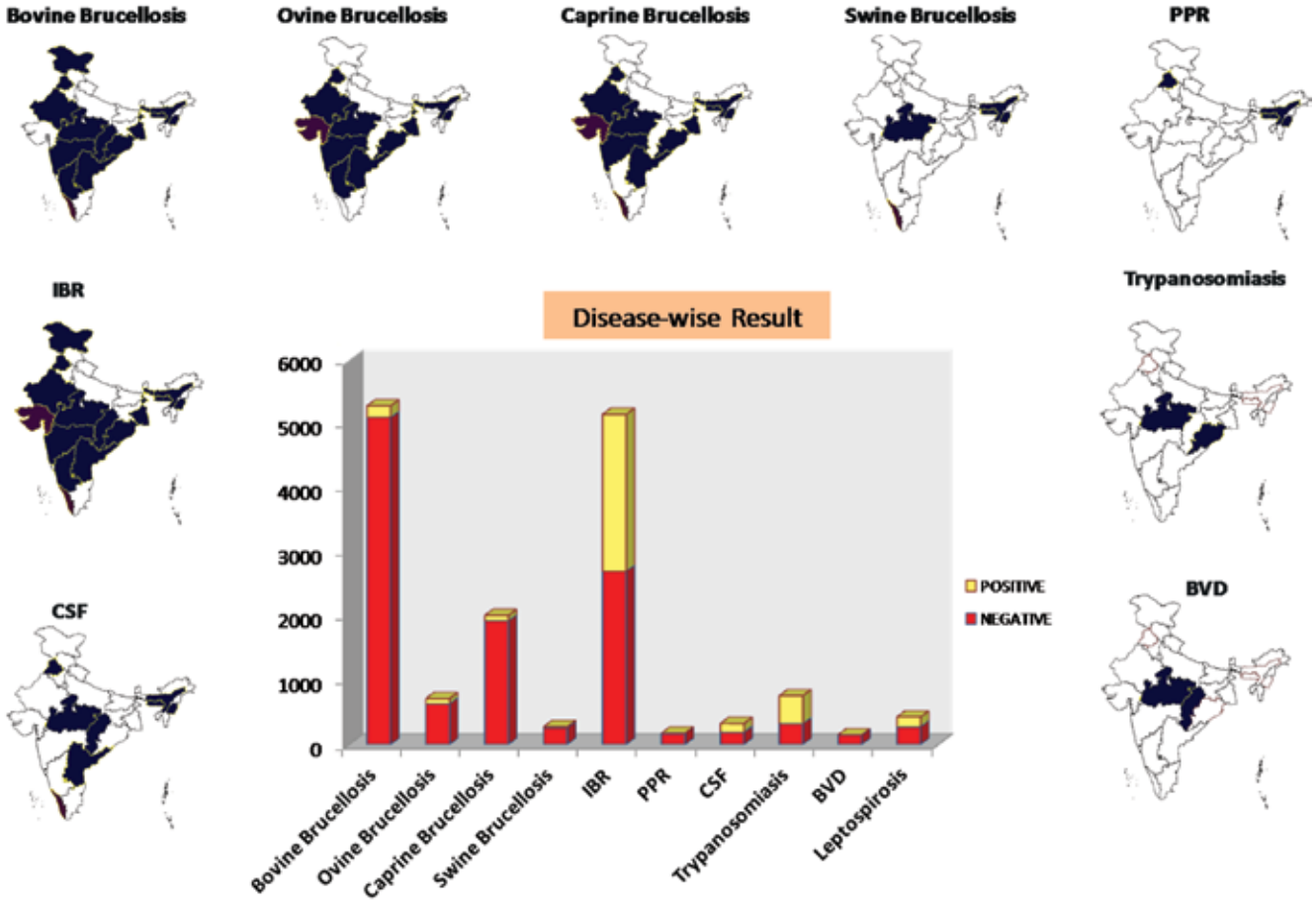


Fig. 55: Shows the state-wise distribution (filled polygons) of samples used for screening against various diseases. Graph below shows disease-wise results.

Grant-In-Aid Project

IPC: ANSCNIVEDISOL201200300027 Project ID: OXX02580

National Control Programme on Brucellosis

H. Rahman, R. Shome and M. Nagalingam

A. Development and evaluation of Lateral Flow Assay for brucellosis

The brucellosis lateral flow assay is a one-step immune-chromatographic assay. A lipopolysaccharide antigen (SLPs) prepared from a culture of *B. abortus* S99 is immobilised in a discrete line on a porous nitrocellulose membrane located in the test zone (absorbent pad). The assay utilises a dried detection reagent (colloidal gold particles conjugated with Protein G) deposited within the conjugated pad of the device. Antibodies in the field serum that are specific for *Brucella* attach to the SLPs antigen and stained by binding to the detection reagent. With any sample a red line should appear in the control zone and positive/ negative status of sample is declared based on comparison of test line with control line.

Brucellosis lateral flow assay kits were evaluated for a panel of 400 serum samples (100 each cattle, sheep and goat, pigs and humans). Results in 1st stage of evaluation showed good agreement with RBPT and iELISA results with Kappa coefficient of 0.9, 0.7, 0.8 and 0.4 for cattle, sheep and goat, pigs and humans serum samples respectively (Table 27).

Table 27: First stage evaluation using Kappa Statistics for detection of Brucellosis using LFA

Sl. no.	Species	Com-parison	Total samples	+ve concor-dant	-ve Con-cor-dant	Discor-dant	X^2 value	P value	Un-weighted kappa	Linear weight-ed Kappa	Qua-dratic weight-ed Kappa	Results for agreement
1	Cattle	RBPT & ELISA Vs LFA	100	17	80	3	81.928	<0.001**	0.9007	0.9007	0.9007	Very good
3	Sheep & Goat	RBPT & ELISA Vs LFA	100	15	78	7	59.603	<0.001**	0.7682	0.7682	0.7682	Good
5	Pigs	RBPT & ELISA Vs LFA	100	17	77	6	66.015	<0.001**	0.8125	0.8125	0.8125	Very good
7	Human	RBPT & ELISA Vs LFA	100	15	63	22	21.243	<0.001**	0.4388	0.4388	0.4388	Moderate

*Kappa Coefficient of agreement

In second stage evaluation of LFA is done with both serum and blood samples of sample animals (Table 28). Preliminary 100 sample results indicated that improvement and evaluation of LFA test is required for analysis of blood samples.

Table 28: Second stage evaluation of LFA with blood and serum samples

Sl. No.	Place of collection	Species	Total No. of Samples	RBPT and iELISA Positive	Serum LFA Positive	Blood LFA Positive
1	Punjab	Bovine	150	80/150	71/150	Not done
2	Jaipur	Camel	50	02/50	02/50	02/50
	Total		**200**	**82/200**	**73/200**	**2/50**
	Positive Percentage			**41.00**	**36.50**	**4.00**

B) Surveillance of bovine brucellosis using milk ring test under NCPB in Karnataka

A total of 64,818 pooled milk samples collected from 30 districts of Karnataka state were tested in two rounds during the period by Department of Animal Husbandry, Government of Karnataka in liaison with Karnataka Milk Federation (KMF) and monitored by NIVEDI. The overall positive pooled samples revealed 2.55% and all the 30 districts of Karnataka were divided in to 3 categories viz. low (<5%), moderate (5-10%) and high (>10%) prevalence districts. Out of 30 districts under study, 23 districts showed low prevalence while 5 districts were showing moderate and 2 districts have shown high prevalence.

C) Veterinarians views /perception on brucellosis in animals

The survey was conducted in eleven different states (Tamil Nadu, Karnataka, Madhya Pradesh, Uttar Pradesh, Delhi, Chhattisgarh, West Bengal, Assam, Meghalaya, Goa and Rajasthan) to obtain the feedback from veterinarians through structured performa. The total respondents were 383 veterinarians working in various capacities in the departments.

Economically important disease: The veterinarians stated FMD as number one economically important disease followed by H.S., parasitic diseases and fifth most important as brucellosis. This data amply suggest and supports the burden of brucellosis as top five most economic important diseases.

Use of protective measures: The veterinarians were asked use of protective measures while handling animals and 75% of veterinarians stated that they use aprons and 79% have practice of using gloves. Whereas, use of masks and goggles by veterinarians was comparatively less (masks-43% and goggles-37%). Many have stated the non-availability of these items in sufficient quantities in the veterinary hospitals.

Brucellosis in vets and paravets: The other key question asked was whether they have noticed symptoms like fever, joint/ muscle pain and orchitis symptoms in veterinary professionals, paravets, farmers etc. the respondents have stated that they were aware of that 8% of vets and 10% of paravets are getting infected with brucellosis.

View on National policy for brucellosis: Majority of doctors (62%) expressed that the farmers should be given compensation or insurance coverage for brucellosis infected animals. Only half of the respondents were in favor of vaccination (52%) and less percentage (14%) of the doctors expressed increased work load in the hospitals. They were not in favour of vaccination as it is biohazardous. These issues suggest strengthening of man power in the hospitals for routine care of the animals in the hospitals and regular vaccinations in various control programs.

AICRP on ADMAS

NIVEDI is having a strong network of All India Co-ordinated Research Project on Animal Disease Monitoring and Surveillance (AICRP on ADMAS) and it is functional since 1987. It is having 15 collaborating units throughout the country. All the PIs and Co-PIs are extensively working related to animal disease diagnosis, outbreak and disease data reporting, disease forecasting, mapping etc. on different animal diseases especially six bacterial (Haemorragic Septicaemia, Anthrax, Black Quarter, Sheep Eenterotoxaemia, Brucellosis and Leptospirosis), five viral (Infectious bovine rhinotracheitis, Bluetongue, Classical swine fever, Peste des Petits Ruminants and Sheep and Goat pox) and five parasitic diseases (Trypanosomosis, Theileriosis, Babesiosis, Fascioliasis and Amphisomiosis).

The annual Scientists' Meet of AICRP on ADMAS for the period 2011-12 and 2012-13 was held at Bengaluru on 21-22nd July, 2013. All the PIs participated in this meet. Dr. Gaya Prasad, ADG (AH) and Dr. H. Rahman, Director, NIVEDI also graced this occasion.The progress of all the units were reviewed and the experts suggested the future course of activities.

The ADMAS centers were graded based on ten parameters for their work carried out during 2012-13 and the following centers were adjudged as top three centers.

1. The best center	Bhopal, Madhya Pradesh
2. The Second best center	Cuttack, Orissa
3. The third best center	Hyderabad, Andhra Pradesh

● Existing centers ● Newly proposed centers

Location of AICRP Centers

Dr. Gaya Prasad, ADG(AH), ICAR, New Delhi releasing NIVEDI News letter.

20th & 21st Annual Review Meet of AICRP_ADMAS held at NIVEDI, Bengaluru on 2nd July, 2013.

Tribal Sub-Plan (TSP)

Tribal Sub-Plan (TSP) is primarily meant for socio-economic development of tribal people protecting them from social exploitation and bringing them to the main stream activities of the society. TSP was started in NIVEDI, Bengaluru during the year 2011-12 and is implemented through AICRP on ADMAS centres. During the year 2013-14, TSP was implemented through four centres viz., Barapani, Meghalaya; Bhopal, Madhya Pradesh; Jaipur, Rajasthan and Srinagar, Jammu and Kashmir, and were continuing the TSP activities since 2011-12.

The activities of TSP under animal husbandry sector were related to distribution of small ruminants, pigs/ piglets, and poultry to tribal people, supply of mineral mixtures, feed supplements and arrangements of animal health camps in the villages wherein the tribes are having livestock population. More than 500 broiler chicks in Mawbri village and 32 piglets in Umlyngka village along with 100 kg of grower feed were distributed to the selected beneficiaries to start up livestock rearing as a venture to improve their livelihood in Meghalaya. A tribal women self help cooperative group, Mayank Mahila Samuh Jolikheda, has been formed (with help of Kesla Poultry society Sukhtawa, Kesla) for poultry rearing at Jolikheda Village, Kesla block in Hoshangabad district and 8 small-holder broiler poultry units (500 birds each) were established under Madhya Pradesh centre. The Animal health camps were being organized regularly by Rajasthan Centre. Fourteen cattle were distributed to tribal farmers by Jammu and Kashmir centre this programme. Establishment of a separate TSP sub cell at each institute and provision of man power assistance would bring in better results and development tribal farmers in India.

Dr. H. Rahman Director, NIVEDI with TSP beneficiaries in Rajasthan on 11th February, 2014.

Dr. Arnab Sen and Dr. Shankunthala of collaborating center of AICRP_ADMAS with TSP beneficiaries in Meghalaya during Animal Health Camp cum Training of Tribal Farmers on 18-20th January, 2014.

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Patents

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- Suryanarayana VVS, Pradhan P, Isloor S and Shome BR. *A Novel Biomarker based Detection of Bovine Subclinical Mastitis.* (Application No. 3808/DEL/2011, dated 26.12.2011).

Capacity Building / Human Resource Development

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

Sl. No.	Name of Seminar /Workshop /Training	Venue	Duration (Days)	Date
1	Training cum Mid-term Review Meet for PI's of DBT-Network Project on Brucellosis	NIVEDI	1	01.06.2013
2	Training Programme on Field Epidemiology	NIVEDI	3	04.06.2013-06.06.2013
3	20th and 21st Annual AICRP Review Meet	NIVEDI	1	02.07.2013
4	Training on Laboratory Techniques in *Brucella* Research and Diagnosis	NIVEDI	3	01.08.2013-03.08.2013
5	Hands on training-cum-workshop on Assessment of Socio-economic Impact of FMD and its Control in India	NIVEDI	3	16.08.2013-18.08.2013
6	Short Course on Advances in Livestock Disease Informatics and Biostatistics	NIVEDI	10	19.08.2013-28.08.2013
7	Brucellosis Sensitization Training	Meghalaya	1	21.08.2013
8	Brucellosis Sensitization Training	Assam	1	22.08.2013
9	Sensitization Training on National control programme on Brucellosis and PPR to the field veterinarian	AHVS, Goa	1	20.09.2013
10	Training Programme on Data Analysis using SAS (NAIP Consortium) under Strengthening of Statistical Computing for NARS	NIVEDI	5	24.09.2013-28.09.2013
11	Brucellosis Sensitization Training	West Bengal	1	07.10.2013
12	Annual Review Meet of DBT-Network Project on Brucellosis	Veterinary College, GADVASU, Ludhiana, Punjab.	2	29.10.2013-30.10.2013
13	Training Programme on Basic Diagnostic Techniques for Leptospirosis	NIVEDI	3	26.11.2013-28.11.2013
14	Trainers Training Course on Epidemiology, Diagnosis and Control of Haemoprotozoan Parasitic Diseases	NIVEDI	10	10.01.2014-19.01.2014
15	Training programme on Research Methodology, Epidemiology and Bio-Statistics	NIVEDI	3	17.01.2014-19.01.2014
16	Trainers Training Course on Field Epidemiology for Field Veterinarians of Sikkim	NIVEDI	10	22.01.2014-31.01.2014
17	Indo-UK Workshop on Bovine Mastitis	NIVEDI	3	01.02.2014-03.02.2014
18	Brucellosis Sensitization Training	Rajasthan	1	11.02.2014
19	Trainers Training Course on Goat farming: Animal Health Management in Small Ruminants	NIVEDI	10	22.02.2014-03.03.2014
20	Training Programme on Field Epidemiology	NIVEDI	5	24.02.2014-28.02.2014
21	AICRP training programme on Field Epidemiology: Basic Epidemiology & Disease Informatics and Livestock Disease Economics	NIVEDI	5	03.03.2014-07.03.2014

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

Sl. No.	Name of the Seminar / Workshop /Training	Venue	Date	Scientist attended
1	XXIII Indian Veterinary Congress and XX Annual Convention of Indian Association for the Advancement of Veterinary Research and International Conference on Thrust areas in Veterinary Research, Education, Regularly Reforms and Governance for Quality Services to Farmers	IAH&VB and Veterinary College, Bengaluru	16.04.2013-17.04.2013	Dr. D. Hemadri Dr. V. Balamurugan Dr. P. Krishnamoorthy Dr. M. Nagalingam
2	SAARC Agricultural Centre (SAC), Bangladesh Sponsored Training Programme on Molecular Techniques in Diagnosis of Diseases of Farm Animals and Poultry	HSADL, Bhopal	22.04.2013-01.05.2013	Dr. P. Krishnamoorthy
3	Global PPR Research Alliance (GPRA) Meeting	Nairobi, Kenya	28.04.2013-29.04.2013	Dr.V. Balamurugan
4	NDMA-CDC Atlanta Conference on "Emerging and Re-emerging Pathogens and their Bio-risk Management"	India Habitat Centre, New Delhi	07.05.2013-08.05.2013	Dr. D. Hemadri
5	Regional Training Workshop on "Practical Application of Designing Risk based Disease Surveillance in Livestock Value Chains" organized by Regional Support Unit, Food and Agriculture Organization of the United Nations	Chandigarh, India	15.05.2013-20.05.2013	Dr. S. S. Patil Dr. G. B. Manjunatha Reddy
6	One Health Initiative for Developing Network Project on Brucellosis in Collaboration with ICMR, New Delhi and Massey University, New Zealand	ICMR, New Delhi	03.06.2013-04.06.2013	Dr. H. Rahman Dr. R. Shome
7	Field Epidemiology Training Programme	NIVEDI	04.06.2013-06.06.2013	Dr. S.S. Patil Dr. G. B. Manjunatha Reddy
8	NDDB-ICAR Joint Working Group on Evaluation of Brucellosis Diagnostics Protocol	ICAR, New Delhi	05.06.2013	Dr. R. Shome
9	Interactive Session on ISO 9001:2008 Awareness and PDCA (Plan-Do-Check-Act cycle), Quality Management Services	NIVEDI	11. 06. 2013	All Scientists and Administrative Staff of NIVEDI
10	International Training Programme on Bluetongue Vector Identification	TANUVAS, Chennai	01.07.2013-03.07.2013	Dr. D. Hemadri
11	21 days Training Programme on Application of Remote Sensing and GIS in Natural Resource Management.	NBSS and LUP, Bengaluru	04.07.2013-24.07.2013	Dr. M. Nagalingam
12	Annual Conference of Association for Prevention and Control of Rabies in India	Pune, Maharashtra	06.07.2013-07.07.2013	Dr. G. B. Manjunatha Reddy
13	National Seminar on Brucellosis and Leptospirosis organized by Indian Veterinary Association on the Eve of World Zoonoses day	Thiruvananthapuram, Kerala	06 .07. 2013	Dr. R. Shome

14	Overview on Changing and Emerging Trends of Brucellosis in the DBT- Sponsored Workshop on *Brucella* Genomics	Madurai Kamaraj University, Madurai	08.07.2013-12.07.2013	Dr. R. Shome Dr. G.B. Manjunatha Reddy
15	Modelling Pathogen Prevalence and Risk in Livestock Production	AAU, Anand, Gujrat	10.07.2013-13.07.2013	Dr. B.R. Shome
16	Bio-care Mentorship Workshop for Women for the South Zone	Chennai	19.07.2013	Dr. R. Sridevi
17	International Conference on Emerging and Trans-boundary Diseases of Global Importance	Madras Veterinary College, TANUVAS, Chennai	15.07.2013-16.07.2013	Dr. P. Krishnamoorthy
18	Development of a Project on Surveillance of Influenza A Viruses in India, including Wild & Migratory Birds	HSADL, Bhopal	07.08.2013-08.08. 2013	Dr. M. R.Gajendragad
19	ICAR-Sponsored Short Course on Advances in Livestock Disease Informatics and Biostatistics	NIVEDI	19.08.2013-28.08.2013	Dr. P. Krishnamoorthy Dr. R. Sridevi
20	Orientation Workshop on National Surveillance Programme on Aquatic Animal Diseases.	NBFGR, Lucknow	17.09.2013-20.09.2013	Dr. M. R.Gajendragad Dr. K. P. Suresh
21	18th International Symposium on Problems on Listeriosis (ISOPOL)	ICAR Research Complex ,Goa	21.09.2013	Dr. H. Rahman
22	Terminal Workshop on Immediate Technical Assistance to Strengthen Emergency Preparedness for HPAI to India	New Delhi	24.09.2013	Dr. H. Rahman
23	Training Programme on Data Analysis using SAS (NAIP Consortium) under Strengthening of Statistical Computing for NARS	NIVEDI, Bengaluru	24.09.2013-28.09.2013	Dr. P. P. Sengupta Dr. V. Balamurugan Dr. M. Nagalingam
24	14th Bengaluru India BIO-Curtain Raiser Programme	Bengaluru	27.09.2013	Dr. V. Balamurugan
25	International Training on Climate Change Vs Immune System: Tools and Techniques for Studying the Immune System under Changing Environment Sponsored by NAIP	CRC, INSERM, Paris, France	02.10.2013-29.12.2013	Dr. G. B. Manjunatha Reddy
26	2nd National Knowledge Network (NKN) Annual Workshop 2013	Indian Institute of Science, Bengaluru	17.10.2013-19.10. 2013	Dr. M. Nagalingam
27	Mid-term Review Meet-Outreach Programme on Zoonotic diseases	Veterinary College, Mathura, Uttar Pradesh	24.10.2013	Dr. R. Shome
28	8th National Conference on KVK	UAS, Bengaluru	23.10.2013-25.10.2013	All Scientists of NIVEDI
29	Annual Review Meet of DBT-Network Project on Brucellosis	Veterinary College, GADVASU, Ludhiana, Punjab.	29.10.2013-30.10.2013	Dr. H. Rahman Dr. R. Shome Dr. M. Nagalingam
30	International Krishi Mela	UAS, Bengaluru	07.11.2013-11.11.2013	All Scientists of NIVEDI
31	AgrIP 2013 National Workshop on Role of Intellectual Property Rights in Modern Era	ZTM-BPD Unit, South Zone, CIFT, Cochin	15.11.2013-16.11.2013	Dr. R. Sridevi
32	Training Programme on Microsoft Office Suite	ISTM, New Delhi	18.11.2013-22.11.2013	Smt. A. Saranya Shri. L. Gangad-hareshwara
33	National Symposium of Indian Association of Veterinary Pathology and National Symposium on Advances and Applications of Diagnostic Pathology for Disease Management in Livestock, Poultry, Pet, Fish, Laboratory Animal and Wildlife.	College of Veterinary Sciences, OUAT, Bhubaneswar	21.11.2013-22.11.2013	Dr. M. R.Gajendragad Dr. P. Krishnamoorthy

34	National One Health Symposium	New Delhi	26.11. 2013	Dr. M.R.Gajendragad
35	Interactive Workshop with the Veterinarians of KVK	ZPD, Kanpur	29.11.2013	Dr. M.R.Gajendragad
36	Interactive Workshop on Administrative and Finance Matters for the ICAR Institute located in Southern Region	NAARM, Hyderabad, AP	09.12.2013-10.12.2013	Dr. H. Rahman Mr. Riyaz Ahmad Mr. R. K. Babu
37	10th Annual Review Meet of AINP on Bluetongue	S.K.Nagar, Gujarat	14.12.2013	Dr. D. Hemadri
38	Management Development Programme on Financial Issues for the Officers of ICAR	NIFM, Faridabad	16.12.2013-20.12.2013	Mr. R.K. Babu
39	Central Joint Staff Council (CJSC) Meeting	New Delhi	16.12.2013	Mr. N. Narayanaswamy
40	Asia Pacific Congress of Virology (APCV-VIROCON-2013)	Amity University, Noida	17.12.2013-20.12.2013	Dr. V. Balamurugan Dr. M. Nagalingam
41	Training for Internal Auditors as per ISO 9001: 2008	Zero Defect Consultants, Bengaluru	22. 01.2014	All Scientists, STO's and Administration Staff of NIVEDI
42	International Workshop on Bluetongue	VBRI, Hyderabad	30.01.2014-01.02.2014	Dr. D. Hemadri
43	Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, New Delhi Sponsored One Week Training for CPCSEA Nominees	National Institute of Animal Welfare, Ballabgarh, Haryana	03.02.2014-07.02.2014	Dr. P. Krishnamoorthy
44	Krishi Vasant 2014: Agriculture Fair cum Exhibition and Farmers- Scientists Interactive Meet	CICR, Nagpur	09.02.2014-13.02.2014	Dr. P. Krishnamoorthy
45	Joint Orientation Workshop on Emerging and Re-emerging Zoonotic Diseases for Medical and Veterinary Professionals	National Center for Disease Control, Delhi.	10.02.2014	Dr. M.R. Gajendragad
46	ICAR Institute-SAU-State Department Interface Meeting	SMILDA, AHVS, Hyderabad	14.02.2014	Dr. D. Hemadri
47	International Conference on Animal Health and Welfare and the 18th Asian Regional Meeting of the Commonwealth Veterinary Association including Satellite Conferences on Progress in Animal Welfare and Canine Rabies Control	NIANP, Adugodi, Bengaluru	20.02.2014-24.02.2014	Dr. P. P. Sengupta Dr. V. Balamurugan Dr. S. S. Patil Dr. K. P. Suresh Dr. G. Govindaraj
48	DBT_BBSRC PPR meeting FADH PPR Project Consultation and Kit Release	TANUVAS, Chennai	07.03.2014	Dr. V. Balamurugan

Awards/Fellowships/Recognition

1. Dr. H. Rahman,Chairman, Expert Committee forInvestigationand Assess the Incidence of Infectious Bovine Rhinotracheitis (IBR) among Animals in Maharashtra State, DADF, Ministry of Agriculture, Govt. of India, New Delhi.
2. Dr. H. Rahman,Chairman, Expert Committee forInvestigation of Foot and MouthDisease (FMD) Outbreak in Karnataka, Govt.. of Karnataka, Bengaluru.
3. Dr. H. Rahman,Member, Technical Advisory Committee for Monitoring and Supervision of National Surveillance Program for Aquatic Animal Diseases, DADF, Ministry of Agriculture, Govt. of India, New Delhi.

4. Dr. H. Rahman, Member, Expert Committee to review Action Plan on Avian Influenza, DADF, Ministry of Agriculture, Govt. of India, New Delhi.

5. Dr. H. Rahman, Member, DBT Expert Committee for Review cum Selection of DBT CREST Award for the year 2012-13, DBT, New Delhi.

6. Dr. H. Rahman, Member, Management Council and Board of Management, Kerala Veterinary and Animal Sciences University (KVASU) at Pookode, Wayanad, Kerala

7. Dr. H. Rahman, Expert Member, Roundtable on Preparedness to Combat Wildlife Diseases in India.

8. Dr. H. Rahman,Expert Member, Brainstorming Meeting for Establishment of an ICMR-ICAR Centre on Zoonotic MAFSU, Nagpur.

9. Dr. B.R.Shome, Royal Society, London and Department of Science and Technology, GoI jointly sponsored the Workshop on "The use of Molecular Epidemiology and Functional Genomics to Underpin the Development of Novel Interventions to Combat Bovine *Staphylococcus aureus* Mastitis in India" jointly organized by NIVEDI and University of Cambridge.

10. Dr. M. Nagalingam obtained Post Graduate Diploma in Technology Management in Agriculture (PGDTMA)-2013.

11. Dr. P. P. Sengupta and Dr. R. Sridevi has been conferred with "Fellow" award for 2012 by the Society for Applied Biotechnology, Tamil Nadu.

12. Dr. P. P. Sengupta, received a "Certificate of Appreciation" by World Society for Protection of Animals for delivering a Lecture on "Animal Disease Forecasting, Forewarning and Disease Trends" and Participation in National Conference on Animal Disaster Management, Delhi, 17-18th April 2013.

13. Dr. G. Govindaraj, awarded Best Poster on "Discriminative Analysis of Goats Reared in Three Different Agroclimatic Zones of Karnataka" In: 13th Indian Veterinary Congress and XX Annual convention of Indian Association for the Advancement of Veterinary Research and International Conference on Thrust Areas in Veterinary Research, Education, Regulatory Reforms and Governance for Quality Services to Farmers held at KVAFSU, Bengaluru, 16-17th April 2013.

Miscellaneous

Institute Management Committee (IMC)

Name	Designation	
Dr. H. Rahman	Director, NIVEDI	Chairman
Dr. D.M. Das	Director, Department of Animal Husbandry & Veterinary Services, Govt.. of Karnataka	Member
Dr. D. Venkateshwarulu	Director, Animal Husbandry Department, Govt.. of Andhra Pradesh, Hyderabad	Member
Dr. Yathiraj	Dean, Veterinary College, KVAFSU, Bengaluru.	Member
Dr. M. Muddurange Gowda	Village & post Kannamangala Taluk Doddaballapura, Dist. Bengaluru Rural District.	Non-official Member
Mrs.V. Shubha Reddy	No.80 B, Village-Kurubarahally, Taluk Gauribidanur, District, Chikkaballapura-581208.	Non-official Member
Mr. A. Srinivasamurthy	F & AO, IIHR, Bengaluru.	Member
Mr. B. Riyaz Ahmed	AO, NIVEDI	Member Secretary

The IMC meeting of the Institute was conducted on 5.10.2013 and 25.03.2014.

Research Advisory Committee (RAC)

Name	Designation	
Dr. M. P. Yadav	Former Director, IVRI, Izatanagar	Chairman
Dr. H. K. Pradhan	Former Joint Director, HSADL, Bhopal	Member
Dr. D. Swarup	Former Director, CIRG, Makhdoom	Member
Dr. S.C. Dubey	Former Joint Director, HSADL, Bhopal	Member
Dr. Anil Rai	Head, CABI (IASRI), New Delhi	Member
Dr. Mruthyunjaya	Former Director, NAIP, New Delhi	Member
Dr. H. Rahman	Director, NIVEDI, Bengaluru	Member
Dr. Gaya Prasad	ADG (AH), ICAR, New Delhi	Member
Dr. D. Hemadri	Principal Scientist, NIVEDI, Bengaluru	Member Secretary

7th Research Advisory Committee (RAC) meeting of the Institute was held on 1st March 2014. Dr. Rahman, welcomed the RAC Chairman and members and gave a brief account on the new developments, both at the infrastructure (new Building) and the research level. He also discussed about the institute's mandate, in the backdrop of the upgradation of the Project Directorate to the National Institute. Dr. Divakar Hemadri, presented the action taken report before the committee. The Committee discussed the ATR in detail and again stressed the need to conduct the brain storming session for identifying ways and means of collecting quality data for epidemiological studies. Dr. M. R. Gajendragad presented the base paper on database development, disease modelling, forecasting. Scientists of NIVEDI presented the overall achievements under each research projects.

Quinquennial Review Team (QRT)

Name	Designation	
Dr. A. T. Sherikar	Ex-VC, MAFSU, Nagpur	Chairman
Dr. A. K. Gehlot	VC, RVASU, Bikaner	Member
Dr. G. K. Singh	Dean, CVSc, GBPUA&T, Pantnagar	Member
Dr. R. Raghavan	Ex. Prof. & Head, Veterinary College, Bengaluru	Member
Dr. G. Butchaiah	Ex-Dean, RAGACOVAS, Pondicherry	Member
Dr. P. D. Juyal	Registrar, GDVASU, Ludhiana	Member
Dr. V. D. Sharma	Ex-Prof. and Head, CVSc, GBPUA&T, Pantnagar	Member
Dr. M. R. Gajendragad	PS, NIVEDI (PD_ADMAS), Bengaluru	Member Secretary

Institute Research Committee (IRC)

Name	Designation	
Dr. H.Rahman	Director, NIVEDI	Chairman
Dr. M. Rajasekhar	Founder Director, PD_ADMAS	Member
Dr. T. Gopal	Former Director, IAH & VB, Bengaluru	Member
Dr. Gopinath Rao	Prof & Head, Dept of Statistics, UAS, Bengaluru	Member
Dr. Lalith Achoth	Prof & Head, Dairy Economics, KVAFSU, Bidar	Member
Dr. Divakar Hemadri	Principal Scientist	Member Secretary

The Institute Research Committee of the Institute was held on 20th April, 2013. Dr. H. Rahman, Chairman, appraised members about the importance of IRC and need for critical evaluation of the research projects. The scientists of the Institute were presented the achievements of the research projects. A total of six external funded projects, eight institute projects, four inter institute projects, five service projects and one PP projects were presented and evaluated critically.

Institutional Animal Ethics Committee (IAEC)

Name	Designation	
Dr. H. Rahman	Director, NIVEDI	Chairman
Dr. S. G. Ramachandra	Chief Research Scientist, IISc, Bengaluru	CPCSEA Nominee
Dr. Susan Mini Jason	Veterinarian	Link Nominee
Mr. D. Prahallada	Social activist	Non Scientific socially aware member
Dr. Vishwanath Bahagwat	Research Scientist, Himalaya Drug Company	Scientist outside the institute
Dr. M.R. Gajendragad	Principal Scientist, NIVEDI	Member
Dr. Divakar Hemadri	Principal Scientist, NIVEDI	Member
Dr. P.P. Sengupta	Principal Scientist, NIVEDI	Member
Dr.P. Krishnamoorthy	Scientist, NIVEDI	Member Secretary

The IAEC meeting of the Institute was conducted on 28.09.2013 and 11.03.2014. The chairman appraised members about the importance of IAEC and need for critical evaluation of the research projects involving animal experiments with humane care. The Member Secretary presented the overall progress reports and the committee approved six projects presented by scientists that require animal experimentation.

Institute Bio Safety Committee (IBSC)

Name	Designation	
Dr. H Rahman	Director, NIVEDI	Chairman
Dr. M.D. Venkatesha	Inchrage Director, IAH&VB, Bengaluru	Member
Dr. S. G. Ramachandra,	PRS, IISc, Bengaluru	Member
Dr. S. Srinivas	MO, IVRI, Bengaluru	Member
Dr. M. R. Gajendragad,	Principal Scientist, NIVEDI	Member
Dr. (Mrs.) R.Shome	Principal Scientist, NIVEDI	Member
Dr. P. P. Sengupta	Principal Scientist, NIVEDI	Member Secretary

5th IBSC meeting of the Institute was held on 24.3.2014 under the chairmanship of Dr. H.Rahman. The chairman appraised members about the importance of IBSC and need for critical evaluation of the research

projects involving DNA recombinant related work with biosafety. Two project proposals presented by the scientists were approved by the committee.

Distinguished Visitors

1. Dr. S. Ayyappan, Secretary, DARE and DG, ICAR, ICAR, New Delhi.
2. Dr. K.M.L. Pathak, DDG (AS), ICAR, New Delhi.
3. Dr. R. M. Acharya, Ex-DDG (AS), ICAR, New Delhi.
4. Dr. M. L. Madan, Ex-DDG (AS), ICAR, New Delhi.
5. Dr. R.N. Sreenivas Gowda, Ex ViceChancellor, KVAFSU, Bidar, Karnataka.
6. Dr. B. Pattnaik, Project Director, PD_FMD, Mukteswar, Nainital, Uttarakhand.
7. Dr. A. J. Rao, Professor and INSA-Senior Scientist, IISc, Bengaluru, Karnataka.
8. Dr. Vasudevappa, Dean, PGS, UAS, Bengaluru, Karnataka.
9. Prof. Duncan Maskell, University of Cambridge, United Kingdom.
10. Dr. Mark Holmes, University of Cambridge, United Kingdom.
11. Dr. Gavin Paterson, University of Cambridge, United Kingdom.
12. Dr. Ewan Harrison, University of Cambridge, United Kingdom.
13. Prof. Ruth Zadoks, University of Glascow, United Kingdom.
14. Shri. S.K. Varshney, Scientist 'F', and Director, DST, New Delhi
15. Dr. A.K. Srivatsava, Director, NDRI, Karnal.
16. Dr. M. Rajasekhar, Former Director, PD_ADMAS, Hebbal, Bengaluru, United Kingdom.
17. Dr. S.R. Rao, Scientist 'G', Advisor, DBT, New Delhi.
18. Prof. Gaya Prasad, ADG (AH), ICAR, New Delhi.
19. Prof. Manmohan Chabra, Retd. Professor, Dept. of Parasitology, College of Veterinary Science, CCSHAU, Hisar, Haryana.
20. Dr.M.P. Yadav, Former Director, IVRI, Izatnagar, Uttar Pradesh.
21. Dr. A.K. Rawat, Scientist F, DBT, New Delhi.
22. Dr. Peter Mertens, Pirbright Institute, UK.

Staff Position during 2013-2014

S. No	Name	Designation
1	Dr. H. Rahman	Director

Scientific Staff

1	Dr. M.R. Gajendragad	Principal Scientist
2	Dr. B.R. Shome	Principal Scientist
3	Dr. (Mrs) Rajeswari Shome	Principal Scientist
4	Dr. Divakar Hemadri	Principal Scientist
5	Dr. P.P. Sengupta	Principal Scientist
6	Dr. V. Balamurugan	Senior Scientist
7	Dr. S.S. Patil	Senior Scientist
8	Dr. G. Govindaraj	Scientist
9	Dr. K.P. Suresh	Scientist
10	Dr. P. Krishnamoorthy	Scientist
11	Dr. Mohd. Mudassar Chanda	Scientist
12	Dr. (Mrs) R. Sridevi	Scientist
13	Dr. Jagadish Hiremath	Scientist
14	Dr. M. Nagalingam	Scientist
15	Dr. G. B. Manjunatha Reddy	Scientist

Technical staff

1	Dr. Yogisharadhya R	Senior Technical Officer
2	Dr. Awadhesh Prajapati	Senior Technical Officer

Administrative Staff

1	Mr. B. Riyaz Ahmed	Admin Officer
2	Mr. Rajeevalochana	Asst Admin Officer
3	Mr. R. K. Babu	AF & AO
4	Mr. N. Narayanaswamy	Assistant
5	Mr. M. Lakshmiah	Assistant
6	Mrs. A. Saranya	Steno Grade-III
7	Mrs. G.C. Sridevi	LDC
8	Ms. R. Rekha Priyadarshini	LDC
9	Mr. L. Gangadareshwara	LDC

Supporting Staff

1	Mr. Ramu	Skilled Support Staff
2	Mr. H. Shivaramiah	Skilled Support Staff
3	Mr. B. Hanumantharaju	Skilled Support Staff

Promotion

- Dr. B. Ganesh Kumar, Senior Scientist selected as Principal Scientist in NAARM and relieved on 20.07.2013.
- Dr. V. Balamurugan, Senior Scientist and Dr. S. S. Patil, Senior Scientist promoted to the next scale of RGP ₹ 9000/-.
- Dr. G. Govindaraj, Scientist and Dr. P. Krishnamoorthy, Scientist promoted to the next scale of RGP ₹ 7000/-.

Superannuation

- Shri P. Narender, Administrative Officer of the institute was superannuated on 30th June 2013.

Joining

- Shri B Riyaz Ahmed joined NIVEDI on 8th August 2013 as Administrative Officer upon his transfer from NIANP Bengaluru.
- Dr. Yogisharadhya R, Senior Technical Officer joined NIVEDI on 16th December 2013.
- Dr. Awadhesh Prajapati, Senior Technical Officer joined NIVEDI on 1st January 2014.

Revenue

Details of Revenue Generated (2013-14)

S.No	Type Activity	Amount (In ₹ Lakhs)
1	Interest on loans and advances	00.04
2	Application Fee	00.04
3	Interest on short term deposit	24.68
4	Sale of diagnostic kits	26.85
5	Miscellaneous receipts	02.10
	Total	**53.71**

Budget

Statement of Budget Allocation and Expenditure (2013-14)

Major Heads	Plan (₹ in lakh)		Non Plan (₹ in lakh)	
	Allocation	Expenditure	Allocation	Expenditure
Grant-in-Aid-Capital	619.00	618.59	016.00	015.50
Grant-in-Aid-General	273.00	272.39	303.00	302.93
Grant-in-Aid-Salaries	-	-	122.40	121.99
Total	892.00	890.98 (99.88%)	441.40	440.42 (99.78%)

NIVEDI Activities

Dr. S. Ayappan, Secretary, DARE and DG, ICAR reviewing the new NIVEDI building construction at Yelahanka, Bengaluru on 24th January, 2014.

Dr. S. Ayappan, Secretary, DARE & DG, ICAR, visiting PD_ADMAS stall and interacting with scientists at 8th National Conference of KVKs at UAS, Bengaluru on 22nd October, 2013.

Shri Arvind Kaushal Additional Secretary, DARE & Secretary, ICAR, visiting NIVEDI and IVRI stall during 8th National Conference of KVKs at UAS, Bengaluru on 23nd October, 2013.

Dr. K. M. L. Pathak, DDG (AS), ICAR, New Delhi interacting with Director and Scientists of NIVEDI and Officials of NDDB to review the progress of Lab and Administrative building at Yelahanka, Bengaluru on 24th October, 2013.

Dr. K. M. L. Pathak, DDG (AS), ICAR, New Delhi chaired the 8th Project Monitoring Committee on 24th October, 2013.

Dr. K. M. L. Pathak, DDG (AS), ICAR, New Delhi is releasing technical bulletin of NIVEDI on 10th October, 2013.

Dr. K. M. L. Pathak, DDG (AS), ICAR, New Delhi is launching the *EpiNet.*India, an *e*-publication of NIVEDI.

Dr. Gaya Prasad, ADG (AH), ICAR hoisting the flag on foundation day of NIVEDI on 1st July, 2013.

Members of expert team investigation for IBR at Baramati, Maharashtra.

Annual Review Meet of DBT Network Project on Brucellosis held at GADVASU, Ludhiana during 29-30th October, 2013.

Interactive training on Brucellosis_Control Programme at Shillong on 28th August, 2013.

Interactive workshop on Livestock Disease Surveillance for Animal Science Scientists of KVK under ZPD, Kanpur during 28-29th November, 2013.

Members of expert team reviewing outbreaks of FMD in Karnataka.

Participants of Indo-UK workshop on Bovine mastitis held at NIVEDI during 1-3rd February, 2014.

International Woman Day celebration on 10th March, 2014.

Shri T. B. Jayachandra, Animal Husbandry Minister, Govt.. of Karnataka visiting the NIVEDI stall and interacting with the scientist in International Krishi Mela, UAS, Bengaluru on 10th November, 2013.

Hindi Sapthaha celebration at NIVEDI on 16th September, 2013.

Trainers training on Epidemiology, Diagnosis and Control of Haemoprotozoan Parasitic Diseases for Field Veterinarians of Sikkim during 10-19th January, 2014.

Scientists of NIVEDI (Dr. P. P. Sengupta, Dr. V. Balamurugan and Dr. M. Nagalingam) attended Camel Health Camp organised by AICRP on ADMAS Centre, Jaipur at Ugariawas Village, Mujamabad Taluk, District Jaipur, Rajasthan on 12th February, 2014.

Scientists of NIVEDI with Dr. V. Raghurame Gowda, Assistant Director, RPES, Kolar during FMD outbreak investigation at Kolar District in Karnataka.

Dr. D. Hemadri, interacting with farmers of Sukalpet (Sindhanor), Karnataka during BT outbreak investigation on 26th February, 2014.

Shri Aghore Debbarma, Hon'ble Minister for Agriculture and Animal Resources, Govt.. of Tripura inaugurating Livestock Farming and Livestock Disease Awareness at Bamuti, West Tripura (Organized jointly by NIVEDI, Bengaluru and ICAR Research Complex for NE Region, Tripura Centre) on 17th February, 2014 .

Dr. P. P. Sengupta collecting samples from camel suspected for Surra at Ugariawas Village, Mujamabad Taluk, District Jaipur, Rajasthan on 12th February, 2014.

Scientists of NIVEDI (Dr. B. R. Shome, Dr. V. Balamurugan and Dr. M. Nagalingam) and IAH&VB, Bengaluru attended FMD outbreak investigation at Mayiladuthurai district, Tamil Nadu on 11th September, 2013.

AICRP_ADMAS -1987

PD_ADMAS -2000

NIVEDI - 2014

भाकृअनुप
ICAR

हर कदम, हर डगर
किसानों का हमसफर
भारतीय कृषि अनुसंधान परिषद

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(Formarly PD_ADMAS)
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