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ANNUAL REPORT 2015 - 16

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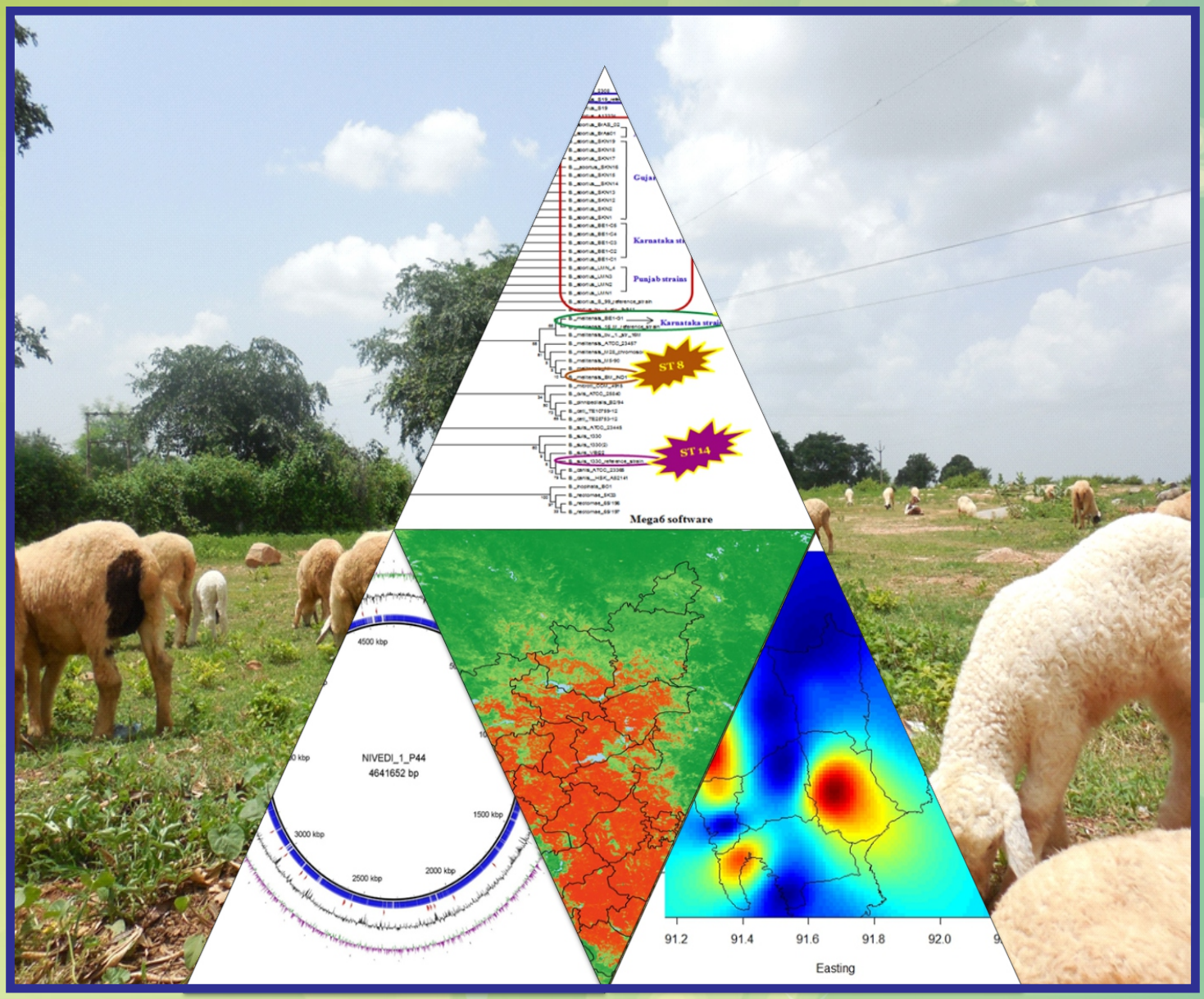
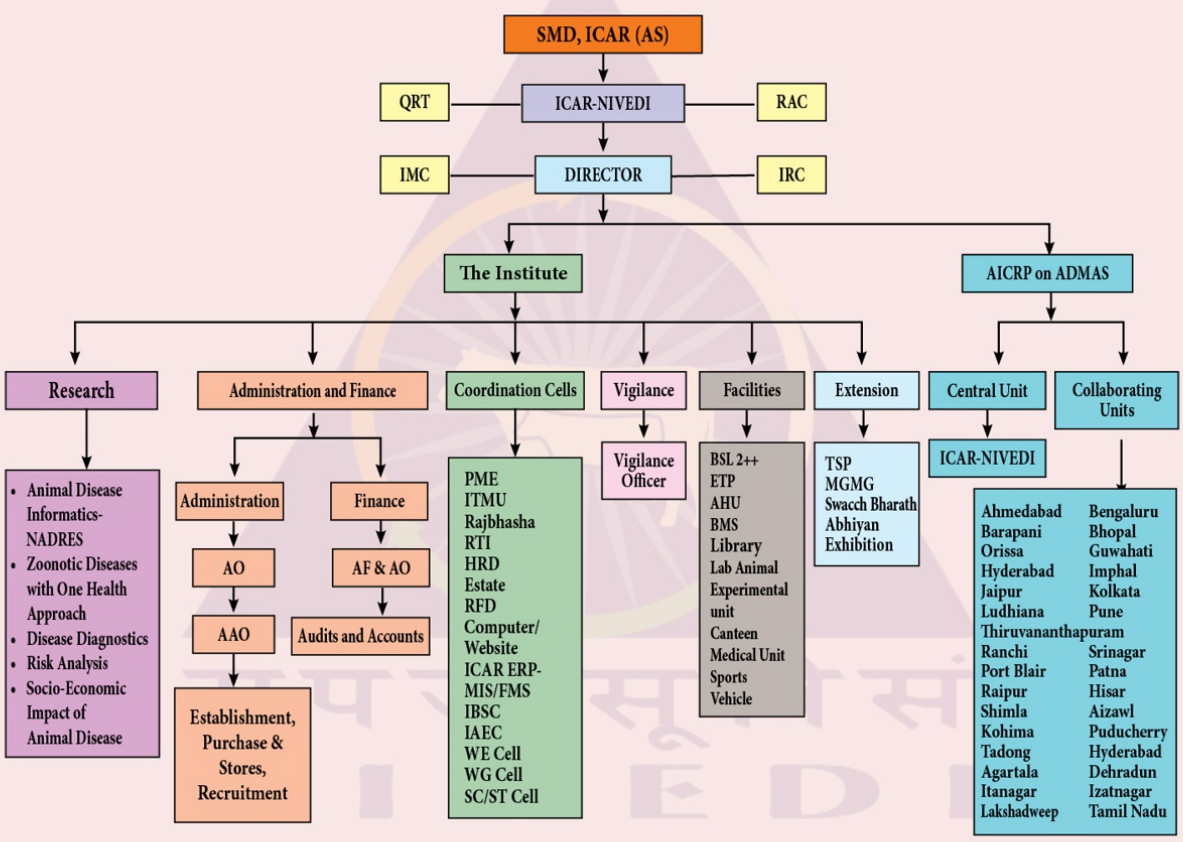


ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (ICAR - NIVEDI)

Post Box No. 6450, Yelahanka, Bengaluru-560064,
Karnataka, India

ORGANOGRAM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Cover Front Page: Salient research outcome in the year 2015-16
Cover Back Page: Nation wide disease outbreak maps 2015-16

Printed at:
PRECISION FOTOTYPE SERVICE
Murugeshpalya, Old Airport Road,
Bengaluru.
Mob : 9845044311

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Acknowledgement

The Director and staff of ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (ICAR-NIVEDI) express their whole-hearted gratitude to Dr. Trilochan Mohapatra, Secretary, DARE and Director General, ICAR for his constant guidance and support.

ICAR-NIVEDI family is also thankful to Dr. H. Rahman, Deputy Director General (Animal Science) and Dr. Ashok Kumar, Assistant Director General (Animal Health) for their generous cooperation, encouragement and support.

Our sincere thanks also to the Director/Head of ICAR institute located in Bengaluru, viz., ICAR-NIANP, ICAR-NBAIR, ICAR-IVRI, ICAR-NBSSLUP, ICAR-NDRI, ICAR-IIHR, KVAFSU, Karnataka Veterinary Council, Director i-AIM, DIG-CRPF and also other Institutes/ organisations for their vital logistics support and co-operation from time to time.

The institute convey sincere thanks to all the principal investigators of AICRP on ADMAS and related state Animal Husbandry Department and Universities for their valuable inputs and cooperation. At the last, I sincerely thank all the staff members of ICAR-NIVEDI for their cooperation.

'Jai Kisan Jai Vigyan'

Jai Hind!

(B R Shome)
Director (Acting)

Executive Summary

During year 2015-16 nine new projects have been initiated including five institutional, three external and one PPP mode projects. Epidemiological survey was carried out on different viral, bacterial and parasitic diseases viz.. IBR, PPR, trypanosomosis, BT, PRRS, TTV, CSF, MCF, capripox, brucellosis, leptospirosis, fasciolosis, mastitis, HS, anthrax etc., This year fasciolosis, reported highest outbreak with 1014 numbers followed by FMD, trypanosomosis, HS, babesiosis, PPR, black quarter, sheep and goat pox, rabies and BT. A total 3257 outbreaks originating from 30 states of 13 major livestock diseases reported to NADRES. Animal disease forecasting was done and circulated to different states and union territories 2 months in advance.

The HS outbreak occurred in Assam was mapped and heat map was also prepared. Risk map for HS in Karnataka was generated using remote sensed variables and showed good correlation with past outbreaks. Out of 29 samples collected from HS suspected cases from elephants, sheep, goat and buffalo from Odisha and Madhya Pradesh, one isolate was obtained. A total of 40 Yak and 160 sheep nasal samples were collected from NE region and processed for isolation of which four *M. haemolytica* were recovered and identified by biochemical test. Along with seven *Pasteurella multocida* type "A" were also isolated.

The outbreaks of HS and FMD occurred in Assam, Karnataka, Kerala and Tamil Nadu, during 2009-14 were mapped and analyzed. The cluster analysis for HS and FMD outbreak showed occurrence of different clusters for HS and FMD and number overlapping of outbreaks observed in Karnataka, Kerala and Tamil Nadu. The estimated average mortality loss due to HS remained Rs. 5,318/- per animal in indigenous cattle, and Rs. 37,000/- in crossbred animal whereas the same found in local buffalo Rs. 19,681/- and in upgraded buffalo Rs. 51,250/-. An economic analysis revealed that total visible loss projected due to FMD in the country during 2015-16 was Rs.721.41 crore.

During anthrax outbreaks investigation, a total of 32 samples were collected from bovines, elephants, sheep and goats from Odisha and Karnataka. Out of which 12 *Bacillus anthracis* isolates were isolated. Risk map for anthrax in Karnataka region was developed based on RS parameters (NDVI, LST, NDWI, NDMI) using 337 anthrax outbreak data from 2000-14 in Karnataka. Many field outbreaks of sheep and goat pox were attended and diagnostics service was provided.

A duplex PCR was standardized for rapid identification and detection of methicillin resistant *Staphylococci* (MRSA, MR-CoNS) targeting genus specific primer and methicillin resistant determinate (*MecA*) primer. A preliminary investigation of antibiotic resistance in livestock in Meghalaya and Assam found that 41% *E.coli* ESBL/AmpC/MβL producer with CTXM-IV was detected as most common determinant among cattle, pig and poultry.

Based on epidemiological study of avian influenza (AI) in Kerala, the possible source of introduction of AI might be the scavenger birds or migratory birds. The spatial map on correlating AI outbreaks (2006-15) and poultry population density in different district was plotted. The disease outbreak data for avian influenza during 2006-15 were analyzed using remote sense, meteorological, anthropometric, cultural and management risk variables.

Twenty PPR outbreaks data from sheep and goat occurred in Madhya Pradesh and Karnataka was analyzed using clinical PPR score card which revealed outbreaks mild to severe form of the disease. The phylogenetic analysis of the N & F gene sequences of PPRV from NE region of India revealed circulation of lineage IV virus. Further 48.86% PPRV antibodies recorded among goats in NE region.

Sero-epidemiology study of NE region, PRRS showed that Mizoram with high prevalence rate (47.71%) followed by Assam (1.63%). Questionnaire was also developed for understanding epidemiology

of PRRSV to identify risk factors was prepared and field validated. Out of 141 serum samples collected from pigs of different NE states only two samples from Mizoram found positive for TTSuV antibodies. Out of 382 samples collected from pigs of different NE states, Arunachal Pradesh showed highest positivity with 77.77% followed by Mizoram (74.5%), Meghalaya (73.5%) and Assam (62.81%) for CSFV. 1.5% seroprevalence was recorded for Bovine Viral Diarrhea virus (BVDV) among ruminants in Nagaland and Manipur. BVDV 1 and BVDV 2 were found prevalent.

During this year 5883 bovine sera samples across 19 states were screened for IBR and an overall 20.81% sero-prevalence was recorded. Chhattisgarh showed highest prevalence with 50.54%. In another study in NER out of 1097 bovine sera samples 20.78% showed positive with Manipur recording highest prevalence with 24.75%.

A total of 151 outbreaks of bluetongue (BT) from four southern states namely Andhra Pradesh, Karnataka, Telangana and Tamil Nadu were recorded and 459 clinical samples collected from the BT outbreaks of nine districts of Karnataka and 94 isolates were recovered in KC cells from the clinical samples and these were belonged to BTV serotype 1, 2, 10, 16 and 23. In a sero-survey in NE region out of 481 serum samples from goat 227 were found positive for BTV.

A total of 7149 serum samples of cattle, buffalo, sheep, goat and pig from 15 states of the country were sero-screened and an overall 3.72% were found positive for presence of anti-brucella antibodies. The Fluorescent Polarization Assay (FPA) for brucellosis has been developed and used for differentiation of vaccinated and infected animals (DIVA). In another study it was observed that commercially available cELISA can be used only beyond 60 days post vaccination as a DIVA test, whereas indigenously developed FPA test can be used from 21 day post vaccinated animals for DIVA. In MLST typing field *Brucella* isolates showed the close genetic similarity with *Brucella* vaccine strain, *B. abortus* S19 and antigenic strain, *B. abortus* S99. Latex agglutination test using rBP26 has been developed for sero-diagnosis of brucellosis.

Out of 300 human serum samples with PUO (Pyrexia of Unknown Origin) cases showed 38% positive cases for the presence of *Leptospira* antibodies with major *Leptospira* serogroups mainly Australis, Bankinang, Tarassovi, Icterohemorrhagiae, Pomona etc., when screened in MAT. Whereas on testing of 125 random livestock serum samples, 36.8% showed sero-positivity in MAT.

Two hundred and sixty human sera samples with PUO cases were screened for both IgG and IgM antibodies against *Toxoplasma gondii* which revealed 28.38% and 2.69% sero-positivity against IgG and IgM specific antibodies respectively. During this period, 381 *Lymnea* sp. snails were collected and screened for the presence of *Fasciola* infection by PCR and 5.77% found positive. Bengaluru Rural districts showed highest positivity with 13.10%. Besides, out of 932 bovine sera samples were collected from Karnataka, West Bengal, Tamil Nadu and Manipur, an overall 43.34% samples found positive for presence of antibodies against surra when tested with recombinant MAb based ELISA. One application for patenting of antigen detecting double antibody sandwich ELISA was filed.

During this period a total of 10753 sera samples from different species of animals were received and catalogued from 23 centers of AICRP on ADMAS. A database framework for the surveillance of aquatic animal disease is under progress. The design document for the same has already been completed.

About ICAR-NIVEDI

ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), (Formerly, Project Directorate on Animal Disease Monitoring and Surveillance, PD_ADMAS) under the Indian Council of Agricultural Research (ICAR), a pioneer research institute in veterinary epidemiology is carrying out disease surveillance, monitoring and analysis of livestock diseases in India through 32 collaborative centers of AICRP onanimal disease monitoring and surveillance (AICRP on ADMAS) located in different states of the country.

The AICRP on ADMAS initiated by the ICAR, made a humble beginning during the VII five-year plan and became fully functional in 1987 with establishment of four Regional Research Units (RRUs) at Bangalore, Hyderabad, Pune and Ludhiana. The Central Coordinating Unit (CCU) was established at the Institute of Animal Health and Veterinary Biologicals, Bengaluru to co-ordinate research activities of the regional units. In the VIII plan, the institute was strengthened with support of ICAR and European Union by taking up the major responsibility under National Project on Rinderpest Eradication (NPRE) involving 32 state level diagnostic/disease investigation laboratories in the country. On 1st April 2000 (during the IX plan), the CCU was given the status of Project Directorate and named as 'Project Directorate on Animal Disease Monitoring and Surveillance (PD_ADMAS)' with ten collaborating units under AICRP_ADMAS component. In the X and XI Five year plan period, five more collaborating units were added for providing impetus to a nationwide animal disease monitoring and surveillance.

Appreciating the contributions made by the Directorate to country's livestock health sector and the need to strengthen the effort, the council rechristened PD_ADMAS as 'National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI)' on 25th October 2013 (XII plan period) with its exclusive campus at Bengaluru. Further, during the same plan period, 17 additional collaborating units covering component totaling to 32 collaborating units for providing the needed impetus to a strong nationwide animal disease monitoring and surveillance network.

ICAR - National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), a pioneer research institute under Indian Council of Agricultural Research (ICAR) has been entrusted to conduct R&D in the field of veterinary epidemiology and surveillance of economically important livestock diseases in the entire country, its role is extremely pivotal for developing models for animal disease forewarning, forecasting, economic impact, risk assessment, and need based animal disease diagnostics. The institute has developed various technologies covering both products and processes and some of them are marketted and/or patented /copyright protected, which are being utilized by various institutes/organizations and different stakeholders in the country. The role of this institute in the eradication of Rinderpest disease in India and development of National Animal Disease Referral Expert System (NADRES) - interactive software for forecasting are noteworthy. The institute conducts various training programmes related to basic epidemiology, sampling frame and sampling techniques, outbreak investigation, research methodologies, disease diagnosis protocols for various stakeholders associated with animal healthcare. Further, NIVEDI envisions to provide newer direction to undertake in-depth R&D activities on epidemiology of emerging and re-emerging, transboundary animal diseases to others involved in the sub-sector in the country, leading finally to prevention, control and eradication of the diseases for achieving animal welfare and safer animal - human interface under one health approach.

Mandate and objectives of the Institute

Vision

Achieving freedom from animal diseases, animal welfare, food and nutritional security through healthy foods of animal origin, poverty alleviation and economic growth of rural India.

Mission

Capacity building in frontier areas of Veterinary Epidemiology dynamics of animal diseases including zoonoses and animal healthcare intelligence.

Focus

- Improving disease monitoring and surveillance through development of pen side diagnostics
- Risk assessment for occurrence of economically important animal diseases
- Adapting strategies to improve animal disease data quality
- Understanding the threat from animal diseases in the background of climate change and globalization
- Developing early warning system and disease modelling/ forecasting
- Understanding economic impacts of animal diseases and the management strategies
- Promoting innovations and improving human resource capacity

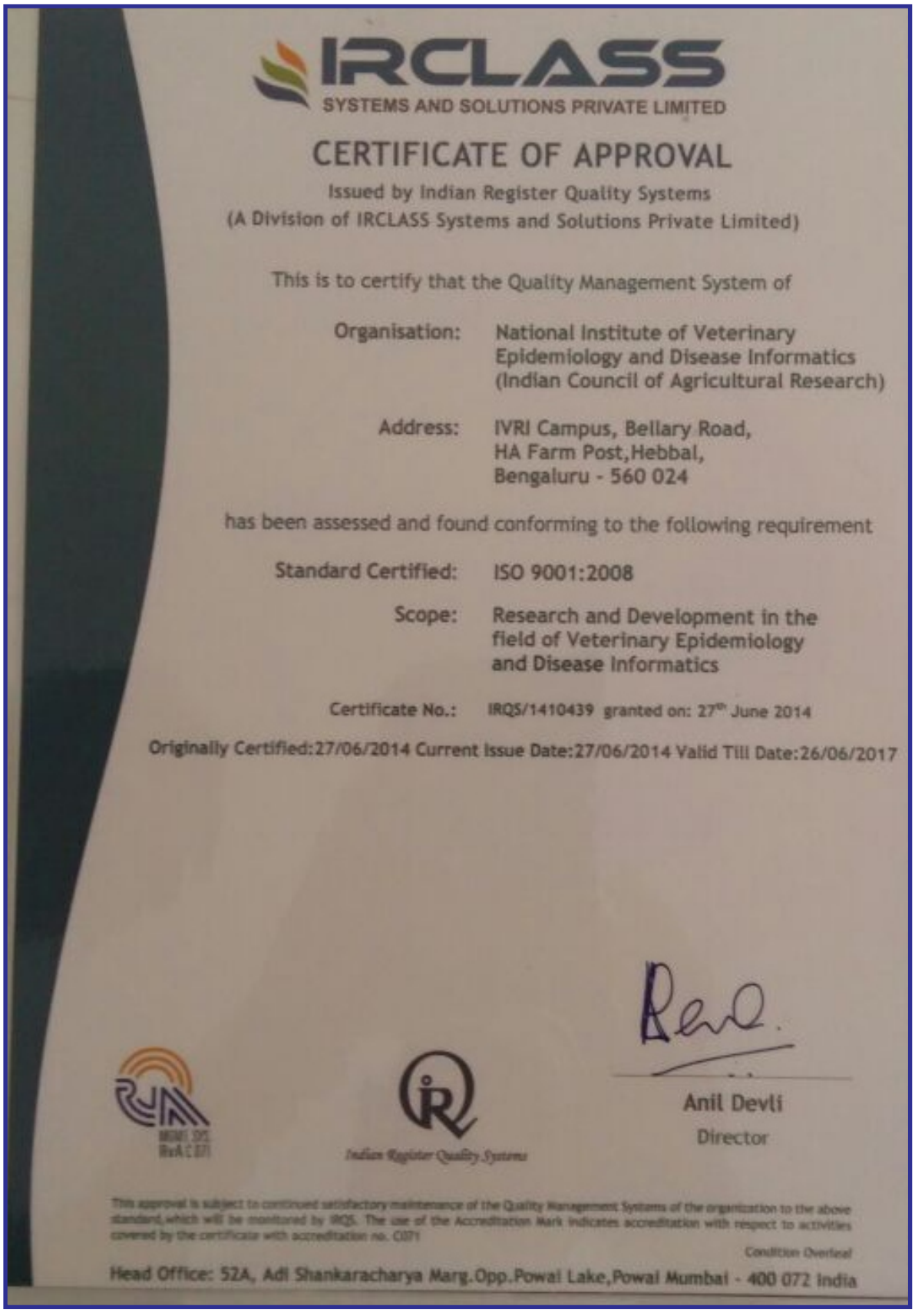
Research mandates of ICAR-NIVEDI

- Research and development on Animal disease informatics and epidemiology
- Understanding specific disease process for rational development of diagnostics and strategic control measures for animal diseases including zoonosis
- Forecasting and forewarning of economically important animal diseases and
- Economics of animal diseases and health care measures

Mandate of AICRP on ADMAS

- Strengthening of National Livestock Serum Repository
- Effective updating of NADRES with active disease data climatic and non-climatic risk factors
- Surveillance of diseases/pathogens of companion, lab and wild animals
- Analysis on economic losses due to animal diseases and the control measures adopted for their management
- Sero-monitoring of animal diseases based on sample frame
- Investigation of endemic, emerging and re-emerging animal disease outbreaks using innovative technologies

ISO 9001:2008 Certification



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Scope: Research and Development in the field of Veterinary Epidemiology and Disease Informatics

Certificate No.: IRQS/1410439 granted on: 27th June 2014

Originally Certified:27/06/2014 Current Issue Date:27/06/2014 Valid Till Date:26/06/2017

Anil Devli
Director

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INSTITUTE RESEARCH PROJECTS

IPC: ANSCNIVEDISIL201500500068 Project ID: IXX12238

Development of Geographic Information System (GIS) enabled Early Warning System (EWS) for detection of Avian Influenza infection using Remote Sensing (RS)

K P Suresh, G S Desai, Md. Mudassar Chanda and R Sridevi

The Avian Influenza disease outbreak data was collected from 2006 to 2015.The total number of outbreaks observed were 102 in different locations of states *viz*. Andhra Pradesh, Assam, Chandigarh, Chhattisgarh, Gujarat, Kerala, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Orissa, Sikkim, Tripura, Uttar Pradesh and West Bengal.

The explanatory variables considered for modelling are remote sensing variables, meteorological variables, anthropometric, environmental variables and management risk variables.

Remote Sensing variables: Normalized Difference Vegetation Index (NDVI), Normalized Difference Water Index (NDWI), Normalized Difference Moisture Index (NDMI) & Land Surface Temperature(LST).

Meteorological variables: Rainfall (mm) and lag period for 1 month, 2 months and 3 months before the date of outbreak occurrence, temperature (Min and Max), occurrence of drought and floods (since 1year).

Anthropometric environmental variables: Distance from major cities (Km), Distance from highways (Km), Distance from Road (Km), Distance Railways (Km), Distance from rivers (Km), Distance to the nearest Lake and wetland/Beel, Terrain/ Elevation (meters), Poultry density and Population density.

Management risk variables: Confinement, visitors in the farm, shared equipment, open water source, near farm infection, backyard poultry, wild bird contact, other livestocks in farm, hygiene status of farm workers, disinfection of transportation vehicles, disinfection of farm and appropriate disposal of dead poultry.

IPC: ANSCNIVEDISIL201500500069 Project ID: IXXI12456

Identification of ecological risk factors for occurrence of Anthrax in India

Md. Mudassar Chanda, D Hemadri, P P Sengupta, K P Suresh, R Sridevi and S B Shivachandra

Anthrax is mainly a disease of herbivores and humans contract the disease directly or indirectly from animals or animal products. It is critical to understand the ecological risk factors responsible for the occurrence of anthrax in India. Thus, the present project aims to understand the intrinsic and extrinsic factors (climate) influencing the occurrence of anthrax outbreaks and to identify risk factors for effective control and prevention of anthrax in India. A number of suspected anthrax outbreaks occurred in Karnataka and Odisha states among domestic and wild animal population were investigated. The clinical materials were collected from dead/live animals as well as from environment for diagnosis of anthrax. All the clinical specimens were processed as per standard conventional and molecular methods. The bacterial isolates of *Bacillus anthracis* were confirmed by growth characteristics, staining and specific PCR assays. The details of outbreaks investigated, sample screened and diagnostic results are indicated in Table 1

Table 1: Epidemiological investigation of suspected anthrax outbreaks and diagnosis

SN	Place of disease occurrence	State	Host species affected	Number of clinical samples screened	Clinical sample type	Diagnosis of anthrax (*Bacillus anthracis*)		
						PA-PCR	Cap-PCR	Isolation
1	Bellary	Karnataka	Sheep & Goat	6	Blood	-	-	-
2	Chamaraj nagar	Karnataka	Cattle	4	Blood	+	+	2
3	Baripada Forest area	Odisha	Elephants	14	Blood/heart/ Liver/spleen	+	+	6
4	Simlipal Tiger Reserve area	Odisha	Elephants	2	Blood	+	+	2
5	Kashipur	Odisha	Bullock	4	Blood/ soil	+	+	1
6	Kashipur	Odisha	Cattle	2	Dried beef	+	+	1
Total samples screened				32	Total isolated *B. anthracis*			**12**

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, R Shome and P Krishnamoorthy

A Duplex PCR was standardized for rapid identification and detection of Methicillin Resistant Staphylococci (MRSA & MR- CoNS) targeting *Staphylococcus* genus specific primer (842 bp) and Methicillin resistance determinant *(mec*A) specific primer (293 bp) using ATCC Methicillin Resistant *S. aureus* (MRSA) reference strain (ATCC 43301) (Fig 1). A total of 112 nasal samples were collected from different livestock (cattle, pig, sheep and goat) and 14 hand swabs were collected from animal handlers from different farms located in Karnataka, Assam and Meghalaya. Duplex PCR assay identified a total of 110 isolates as *Staphylococcus* spp from livestock samples and 8 *Staphylococcus* spp. from animal handlers. Of these, three isolates were found to be *mecA* positive, identified as *S. epidermidis* by laboratory standardized Multiplex PCR assay. SCC *mec* Typing found two isolates to be of Type V and the other was non-typeable. Further a total of 9 nasal swabs collected from calfs in Doddaballapur district found one isolate to be *mec*A positive *Staphylococcus* spp. identified as *S. epidermidis*. Further, phenotypic detection by antibiotic sensitivity test (ABST) by Disc Diffusion test as per CLSI guidelines using ATCC 25931 as quality control standard showed highest resistance patterns for ampicillin and penicillin followed by vancomycin and methicillin amongst cattle.

Subsequently, all the Methicillin Resistant *S. epidermidis* found in the study (n=4) were further genotyped by Multi Locus Sequence Typing (MLST) analysis which allowed detection of a common clonal strain ST-179 from Goat & Pig handler originating from two geographical locations (Table 2). For human hospital set up a total of 70/ 96 isolates from diverse human clinical samples (pus, sputum, urine) KIMS hospital, Bangalore were found to be antibiotic resistant revealing presence of 18 different resistance genotypes. Further for *in vivo* evaluation of modified and new molecule for antibacterial activities and a representative of arul-alkyl lysines designated NCK-10; a novel glycopeptides YV4465; a maleic anhydrate based novel cationic polymer and a new class of semisynthetic glycopeptides antibiotics were the molecules tested and found promising molecules for antibacterial activities in murine model of specific infections.

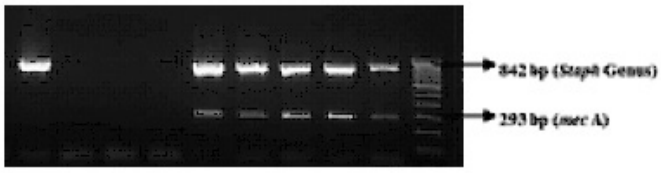


Fig 1: Representative figure of Duplex PCR showing simultaneous amplification of *mec A* and 16SrRNA

Table 2: MLST information of Methicillin Resistant *S. epidermidis*

Sample ID	Location	Source	Sequence Type (MLST)	SCC *mec* typing
H3OW	Gouribidinaru	Pig handler	57	Type V
KN4W	Hosur	Goat	179	Untypeable
PHN16W	Tumkur	Pig handler	179	Untypeable
GCW	Doddabalapura	Calf	130	-

IPC:ANSCNIVEDISIL201200200026 Project ID: IXX07978

Economic analysis of haemorrhagic septicaemia in cattle and buffaloes in selected endemic states of India

G Govindaraj and P Krishnamoorthy

Madhya Pradesh state was selected for assessing the impact of haemorrhagic septicaemia in cattle and buffaloes during the year 2015. Multistage sampling technique was followed in the present study for conducting primary survey. The two highest HS occurring districts viz., Chhindwara and Jabalpur were selected for the survey. In each of the selected district, three blocks were selected for the survey. In each of the selected blocks, the villages were selected based on the secondary data on the occurrence of outbreaks. Around 20 HS outbreak affected villages were surveyed to assess the loss due to HS. The study considered mortality and morbidity parameters like milk loss, draught power loss, treatment cost, veterinarian fees and increased labour engagement cost. The estimated average mortality loss per animal in indigenous cattle and crossbred cattle was Rs.5318 and Rs.37000 respectively. In local and upgraded buffaloes, the estimated average mortality loss per animal was Rs.19681 and Rs.51250, respectively. Besides mortality loss, draught power loss, milk loss, treatment cost and labour cost for different species were also assessed.

IPC:ANSCNIVEDISIL201201000034 Project ID: IXX09422

Epidemiology of haemorrhagic septicaemia in livestock vis-à-vis foot and mouth disease in India

P Krishnamoorthy, B R Shome and G Govindaraj

During the period reported upon, time series data on HS and FMD outbreaks from 2009 to 2014 were collected from Assam, Karnataka, Kerala, and Tamilnadu. The HS and FMD outbreaks in all the study states showed decreasing trend from 2009 except during 2013-14 for FMD. The decreasing trend might be due to effective vaccination and preventive measures adopted by State Animal Husbandry departments of these states. The month wise analysis showed increased occurrence of HS during July to September and FMD during October to December months. Further, the geographical coordinates (latitude and longitude) of HS and FMD outbreaks occurred villages were collected and cluster maps were prepared using EpiInfo software version 7, CDC, Atlanta, USA. The cluster analysis showed non pathogenic nature of culture isolates. The occurrence of different clusters for HS and FMD, no overlapping of the outbreaks was observed in Karnataka, Kerala and Tamilnadu. The HS outbreaks occurred in Assam state was mapped and heat map was prepared and shown in Fig 2a & 2b. In Assam, districts affected with both HS and FMD was more compared to other states. In Karnataka, the districts showing more number of HS and FMD outbreaks were Hassan, Chitradurga, Bellary & Chikkaballapur, Gulbarga, Yadgir whereas in Kerala it was in Kollam, Thriuvanathapuram, Thrissur, Kollam and Kozhikode. In Tamil Nadu more HS outbreaks were reported in Kancheepuram and Thiruvannamalai and FMD outbreaks in Cuddalore, Thanjavur, Villupuram districts. The HS prevalence rate was highest in Assam and FMD in Kerala. The mortality rate in HS was high in Assam whereas it was high for FMD in

Tamilnadu (Table 3). The highest case fatality rate was observed in Tamilnadu for HS and Karnataka for FMD. Besides secondary data, nine nasal swabs from pigs suspected for HS were collected and subjected for *Pasteurella multocida* species and type B specific PCR and all samples were found negative. The cultures isolated were subjected to mouse inoculation test, but the mice were not succumbed to the infection

Table 3: Prevalence, mortality and case fatality rate of haemorrhagic septicemia and foot and mouth disease occurred during 2009-14

States	Hemorrhagic septicemia			Foot and Mouth Disease		
	Prevalence rate ($x10^3$)	Mortality rate ($x10^3$)	Case fatality rate (%)	Prevalence rate ($x10^3$)	Mortality rate ($x10^3$)	Case fatality rate (%)
Assam	0.38	0.20	51.14	4.99	0.04	0.73
Karnataka	0.05	0.02	56.62	1.67	0.08	4.99
Kerala	0.17	0.10	60.98	10.58	0.10	0.91
Tamilnadu	0.007	0.006	86.49	3.26	0.14	4.19

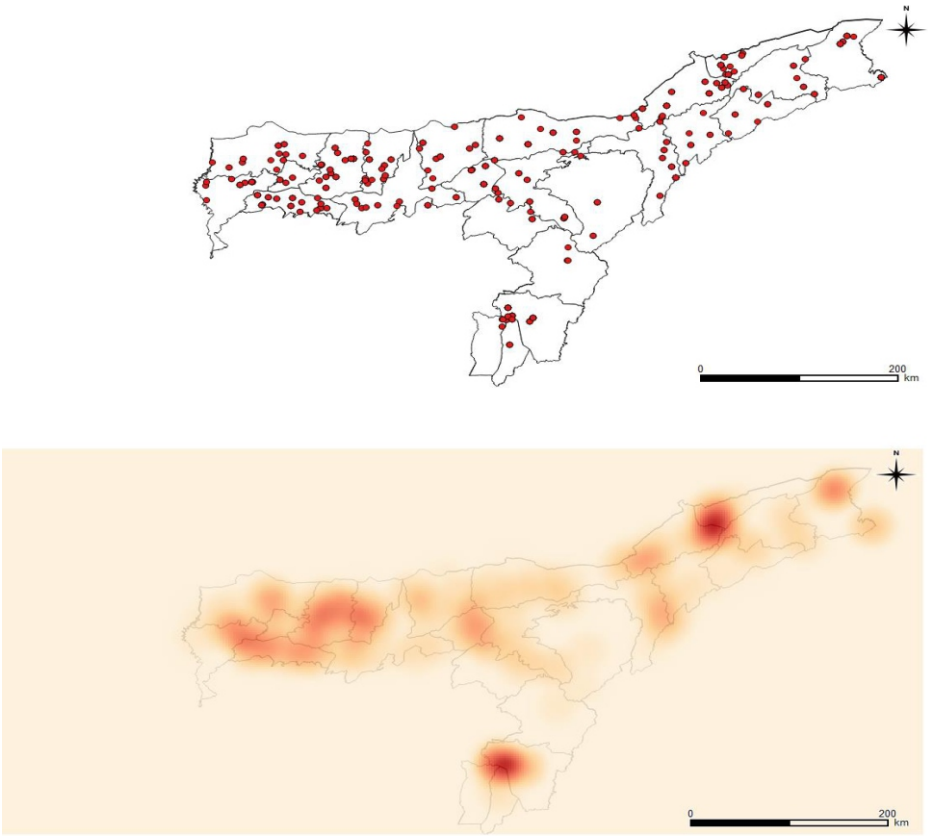


Fig 2 : (a) Haemorrhagic septicemia outbreaks occurred in Assam state during 2009-14 and (b) heat map of HS outbreaks

IPC:ANSCNIVEDISIL201500300066

Project ID: IXX12176

Epidemiology of haemorrhagic septicaemia in India

S B Shivachandra, Md. Mudassar Chanda, J Hiremath, P Krishnamoorthy and R Yogishardhya

Haemorrhagic septicaemia (HS), an acute, highly fatal and septicaemic disease of cattle and buffaloes, is caused by Gram positive bacterium *Pasteurella multocida.* The disease has been recorded in most geographical regions of India. In the present study, an attempt was made to develop a risk map for HS in Karnataka state using remotely sensed variables as potential predictors for disease (Fig 3). The risk map generated showed good correspondence with the past outbreaks. Therefore, risk maps developed using remotely sensed variables may be suitable for informing disease managers concerned with controlling HS by planning vaccination, allocation of resources in high risk areas and also future surveillance of the disease in the region.

During the reporting period, a total of 15 nasal swabs from sheep/goat/buffaloes originated from Madhya Pradesh state and 14 clinical samples (blood, heart, liver, spleen) from dead elephants of Odisha state were screened for presence of *P. multocida* by conventional methods as well as PM specific PCR assay. All the samples were found negative for *P. multocida* except one nasal swab sample from buffalo of Madhya Pradesh found positive in PM-PCR assay with amplification of ~460 bp product. A PCR amplified and cloned N-terminal gene (~2061 bp) encoding for NanB-Nt protein (~94 kDa) of *P. multocida* B:2 in a pET32a vector, was over-expressed in recombinant *E. coli* BL21-CodonPlus (DE3)-RIPL cells. Further, a purified rNanB-Nt protein would be evaluated for its efficacy in detecting HS specific antibodies in sera samples of cattle and buffaloes using indirect-ELISA format. Estimating HS vaccine effectiveness (VE) and identifying its influencing factors is important for HS control in endemic areas. Hence, one of the objectives to estimate the HS VE and to identify the factors that influence VE has been taken up. Towards achieving this objective a needful activities like preparation of questionnaires and their validation has also been initiated.

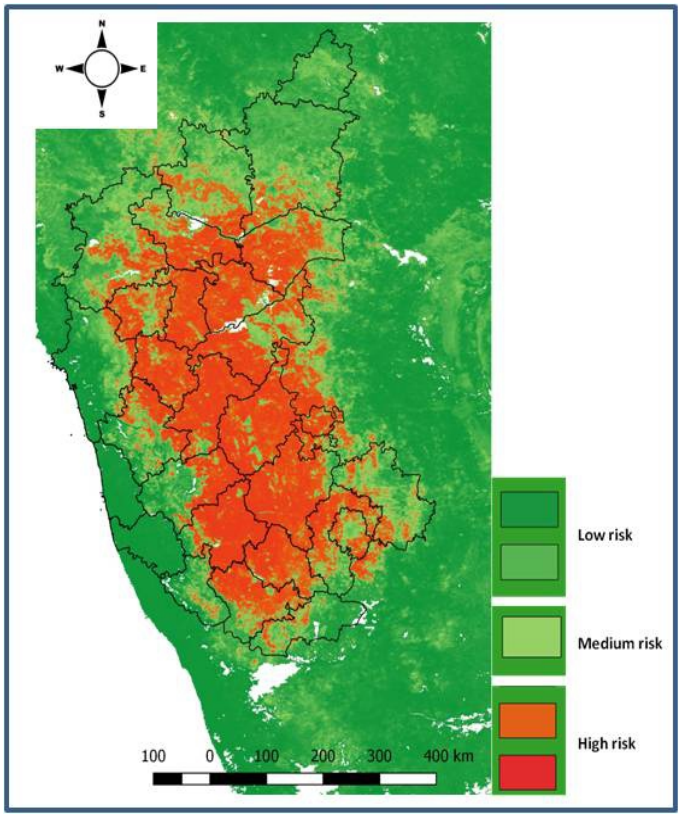


Fig 3: Risk map for haemorrhagic septicaemia using remote sense variables

IPC: ANSCNIVEDISIL201201800042 Project ID: IXX09665

Epidemiology and impact analysis of sheep and goat pox

G B Manjunatha Reddy, V Balamurugan, K P Suresh, D Hemadri, S S Patil and G Govindaraj

A total 3444 pox outbreaks were reported from 2005-2013 from different states in India. Highest numbers of outbreaks were reported from AP with varied number of cases and deaths in outbreak. The number of deaths was directly proportional to number of disease outbreaks and number of attacks in each year. The sheep and goat pox disease outbreaks were more recorded during December to May months.

In field outbreaks, the clinical signs observed were fever (more than 40°C), vesicles, anorexia, loss of body condition, wool loss, nodules in hairless regions of body including the axillae, groin, perineum, ventral surface of the tail, muzzle, udder, teats, around the muzzle and lips and ears were observed. Post-mortem examination revealed typical gunshot wounds/pox lesions in lungs with congestion and consolidation, enlargement of prescapular and mediastinal lymph nodes. Mucosal congestion and sometimes haemorrhages in other visceral organs. The microscopic lesions of oedema, thickening of alveolar septa, mononuclear cell infiltration (MNCs), congestion and various degrees of vascular haemorrhages, necrosis were recorded in the lungs. The skin sections revealed hyperkeratization, epithelial cell proliferation, infiltration of MNCs in the dermis, intracytoplasmic inclusions and necrosis similar to the earlier reports were recorded. Lymph nodes also showed varying degrees of haemorrhages, oedema, follicular depletion and infiltration of inflammatory cells. The *Capripox virus* was confirmed in the clinical samples and cell culture by p32 gene based PCR and expected specific amplification of 237 bp product and confirmed as *Capripox virus* by sequencing. The *Capripox virus* was isolated from the clinical samples (scabs, skin, lungs and swabs) with cytopathic effects such as rounding of cells, clumping and detachment. The phylogenetic analysis of P 32 gene revealed 94.6% to 100 % homology with all the other Indian *Capripox virus* isolates at nucleotide as well as amino acid levels.

IPC: ANSCNIVEDICIL201400600059 Project ID: IXX11154

Epidemiology of Influenza viruses in pigs

G S Desai, S S Patil and N N Barman

About 13 nasal swabs collected from pigs of Aizwal and Mizoram during June 2015 and eight nasal swab samples and blood samples were collected from pig farm in Halebudnoor village, Mandya district, Karnataka for influenza virus detection from nasal swabs. Different oligonucleotide primer sets based on M gene, N gene and HA gene of influenza virus was synthesized for use in RT-PCR. Thirty two numbers of recombinant plasmids with influenza virus (four types) genes along with reverse genetics plasmid vector (pHW2000) were procured from St. Jude Children Res. Hospital, Memphis, TN, USA, under a memorandum of understanding.

IPC: ANSCNIVEDISIL201500200065 Project ID: IXX09659

Risk factors identification and disease severity pattern for PPR in sheep and goats in India

V Balamurugan, G Govindaraj, G B Manjunatha Reddy and R Yogisharadhya

The present scenario of PPR in India warrants the studies to be undertaken on the effect of vaccination and the occurrence of PPR in sheep and goats and also the severity of the disease pattern in different geographical locations under both vaccinated and non-vaccinated conditions. There is a need for the identification of risk factors that influence the incidence of PPR. The comprehensive information on severity and risk factors that influence the incidence of PPR as a whole in India is not available except few. Hence we have developed the methodology (PPR clinical score card) for assessing the severity of the disease pattern during PPR outbreaks in sheep and goats. The severity pattern vary mild, moderate, severe, and very severe. By using this PPR clinical score card, the severity of the disease pattern during PPR outbreaks in sheep and goats in the selected states of India (vaccinated and unvaccinated area) was carried out. Data collected from the PPR outbreaks (n=25) in sheep and goats occurred in Madhya Pradesh (non-vaccinated area) and Karnataka (Vaccinated area) (n=13) was analyzed by using PPR clinical score card with the help of Excel-module to calculate weighted score index to identify the disease pattern. The results revealed that the outbreaks observed were from mild to severe form, with 20 numbers of mild form and five numbers of moderate form of outbreaks in Madhya Pradesh and seven moderate forms, five severe forms and one mild form in Karnataka. Future work is in progress for risk factors identification and identification of the severity of the disease pattern during PPR outbreaks in sheep and goats in other states of India.

IPC: ANSCNIVEDISIL201500400067 Project ID: IXX12421

Epidemiology of Porcine Respiratory and Reproductive Syndrome in India

J Hiremath, D Hemadri, G S Desai, S S Patil, K P Suresh and Md. Mudassar Chanda

Porcine respiratory and reproductive syndrome (PRRS) is an economically important disease of pigs. India had first outbreak of PRRS in the year 2013 and subsequently disease surfaced in 2015. Understanding the epidemiology of PRRS in India is important for developing effective control methods. Hence, the project was taken up with objectives of identifying the risk factors of the disease, assessing the risk of getting PRRS from northeastern India to rest of India and finally to know the seroprevalence in south India. A questionnaire was developed to identify the risk factors and its pilot testing was done in Aizawl district of Mizoram. The major variables in the questionnaire were farm details, clinical signs of PRRS, source and movement of live pigs, proximity of other farms, feed and water sources and biosecurity measures at the farm. The questionnaire based data and clinical samples were collected in Aizawl, Mizoram from 14 pig farmers and few clinical samples (blood for serum & plasma) from pigs with high fever were collected. The questionnaire data from pilot study was compiled (Table 4) and the samples (blood & serum) were analyzed for PRRSV viral RNA and anti-PRRSV specific antibodies. Of 12 serum samples tested 8 were ELISA positive. Pig rearing is a household activity in Aizawl with 2-3 pig per household. Pigs were fed with swill and commercial feed. Majority of the farmers raise piglets holds where the pilot questionnaire data and to finisher, hence purchase of piglets is common.

Seventy percent of the farmers use rented vehicle for pig transport and such vehicles can play significant role in disease spread if proper disinfecting measures are not taken. Improper disposal of carcass can potentially spread the disease and 60% of the farms that dispose dead pigs openly were positive for PRRS.

Although most of the farmers treat sick animals, they neither have an isolation area nor quarantine area in the farm which can be potential risk for PRRS outbreak.

Table 4: Summary of variables and their association with PRRSV status

Variable Name	Value (%)	Number of PRRSV Positive, ELISA (%)	Number of PRRSV Negative, ELISA (%)
Recent abortion (<4 Week)	Yes (33)	50	50
	No (67)	50	50
Clinical signs			
a) Temperature (>104^{0}F=Yes, <104^{0}F=No)	Yes (50)	67	33
	No (50)	33	67
b) Bluing of ear	Yes (67)	50	50
	No (33)	50	50
c) Coughing	Yes (17)	0	100
	No (83)	60	40
d) Respiratory illness	Yes (67)	25	75
	No (33)	100	0
Breeding method	NB (67)	50	50
	AI (33)	50	50
Transport vehicles	Own (33)	50	50
	Rented (67)	50	50
Disposal of dead pigs	Buried (17)	0	100
	Open Disposal (83)	60	40

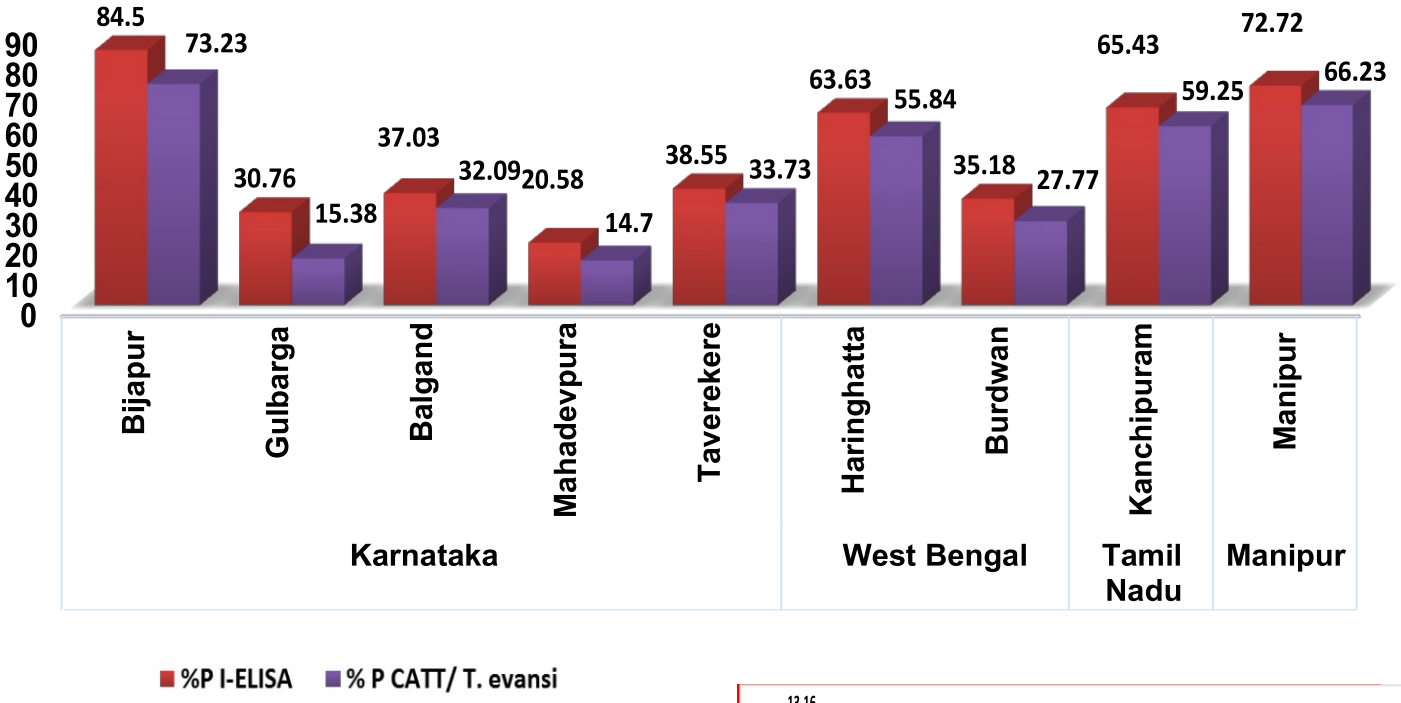
IPC: ANSCNIVEDISIL201100500024 Project ID: IXX07976

Epidemiological study of surra and fascioliosis in animals

P P Sengupta, V Balamurugan, P Krishnamoorthy and S S Jacob

A total of 571 bovine serum samples from Karnataka (282), West Bengal (131), Tamil Nadu (81), and Manipur (77) were screened by ELISA and CATT/*T. evansi*. An overall 310 (54.29%) and 270 (47.28%) samples were found positive for the presence of antibodies of *T.evansi* by ELISA and CATT respectively. State wise prevalence study showed highest sero-positivity of 84.5% in Karnataka followed by Manipur (72.72%), Tamil Nadu (65.43%) and in West Bengal (63.63%). Within Karnataka state, Bijapur showed sero-positivity of 84.5% in ELISA and 73.23% in CATT (Fig 4).

Fig 4: Sero-prevalence of surra in bovine in different states



381 snails (*Lymnea sp.*) were collected from different districts of Karnataka and subjected to PCR for the presence of *Fasciola* infection. Over all 22 (5.77%) were found positive in Karnataka for *Fasciola* infection. District wise sero-positivity for fasciola infection revealed the presence of highest infection in Bengaluru with 13.16%, followed by Chitradurga (11.11%), Tumkur (6.98%), Udupi (6.25%), Coorg (5.71%), Koppal(5.26%), Dakshina Kannada (4.62%), Chikkamagaluru(4.35%), Ramanagara (4%), Uttara Kannada (3.45%), & Shivamogga (2.63%) (Fig 5).

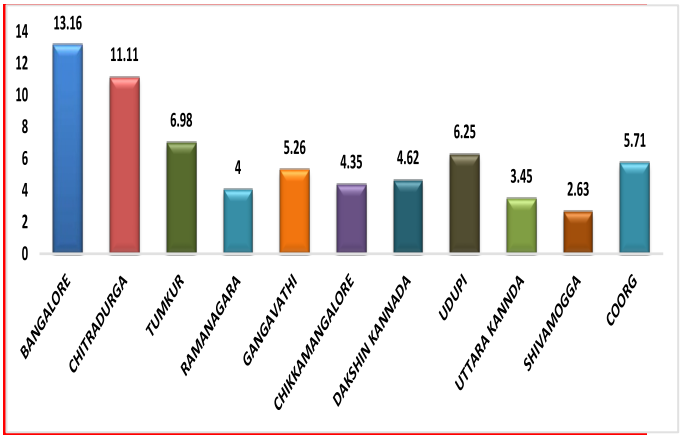


Fig 5: Prevalence of *Fasciola* infection in *Lymnea* sp. snails in different districts of Karnataka.

INTER INSTITUTIONAL PROJECTS

IPC: ANSCNIVEDICOL201300400047

Project ID: OXX02385

Assessment of socio-economic impact of FMD and its control in India

G Govindaraj, S S Patil and K P Suresh (ICAR-NIVEDI)
B Ganeshkumar (ICAR-NAARM), B B Dash, S Saravanan, G K Sharma
(ICAR-PDFMD), R G Bambal (DADF), J Misri (ICAR HQ)

FMD causes huge loss to livestock farmers besides other stakeholders in the livestock value chain. The disease patterns changes in short- as well as in long-run and hence, the impact of the disease has to be assessed on a dynamic basis. Hence, the FMD impact study was extended for 2015-16 covering additional states and districts. Multistage cluster random sampling technique was followed to survey the livestock farms in each of the identified states. Appropriate mathematical models were developed to assess milk loss, draught power loss, treatment cost incurred for the FMD infected animal, cost of extra labour engaged for nursing the animal, mortality loss and loss due to distress sale. During 2015-16 primary survey was undertaken in 11 states viz., Assam, Gujarat, Haryana, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Tamil Nadu, Odisha, Punjab and Tripura to assess the impact. Around 660 villages and 57 panchayats (Kerala) from 49 districts from 11 states comprising 7304 farms were surveyed during 2015. The brief results revealed that the total visible loss due to FMD in the country during 2015-16 was Rs. 721.41 crore.

IPC: ANSCNIVEDICOP201201100035

Project ID: IXX09659

Risk analysis of introduction of Notifiable Avian Influenza (NAI, HPNAI and LPNAI) in India with special referance to risk of NAI through trade and / or non- trade activities

K P Suresh (ICAR-NIVEDI),
D D Kulkarni, S Bhatia, H V Murugkar, and C Tosh (ICAR-NIHSAD, Bhopal)

A risk assessment model has been developed to determine the framework that could lead to the introduction and maintenance of AI virus via the importation of chicken meat, live birds and chicken by-products into the country.

The probability of occurrence of the hazard was carried out using R-PERT software with Monte Carlo simulation. Prevalence data were collected from published reports and their probabilities were calculated using R-PERT software. According to the quantitative risk assessment, the risk of introduction of Avian Influenza virus into India is 24 units (0.88% of import) from Asian countries, 1363 units (0.36%) from European countries, 0.41 units (0.33%) from Australia and 613 units (0.76%) from North American countries. Risk of introducing AI virus is very low due to import of chicken meat, live birds and chicken by-products (<2%).

Retrospective epidemiological studies on HPAI with reference to spatio-temporal pattern and the probable associated risk factors identification

R Sridevi, K P Suresh, A A Raut, P Krishnamoorthy and G Govindaraj

Secondary data on poultry population, human population, area, rainfall, temperature, bird sanctuaries and water bodies in the AI outbreak districts were collected and compiled. Further, distance between the outbreak place and the nearby major cities, major roads / highways, nearby migratory birds destinations were also collected. The spatial map on correlating AI outbreaks and poultry population density and human population density in different districts were plotted (Fig 6). The primary data to assess the economic loss and risk factors was collected from outbreak districts of Kerala viz Alapuzha and Kottayam Based on the epidemiological study of the avian influenza outbreaks in Kerala, the possible source of introduction of the virus might be the scavenger birds –crows or by migratory birds as the migratory birds reaches Kerala backwaters during Nov-Jan season. Based on the epidemiological investigation of avian influenza outbreak in the regional turkeys farm Kollam the source of introduction might be from the previous outbreaks in Kuttanad area or possibly by local birds or by the movement of people /workers. Epidemic curve of AI outbreak in Turkeys farm plotted based on the date of onset and number of birds affected.

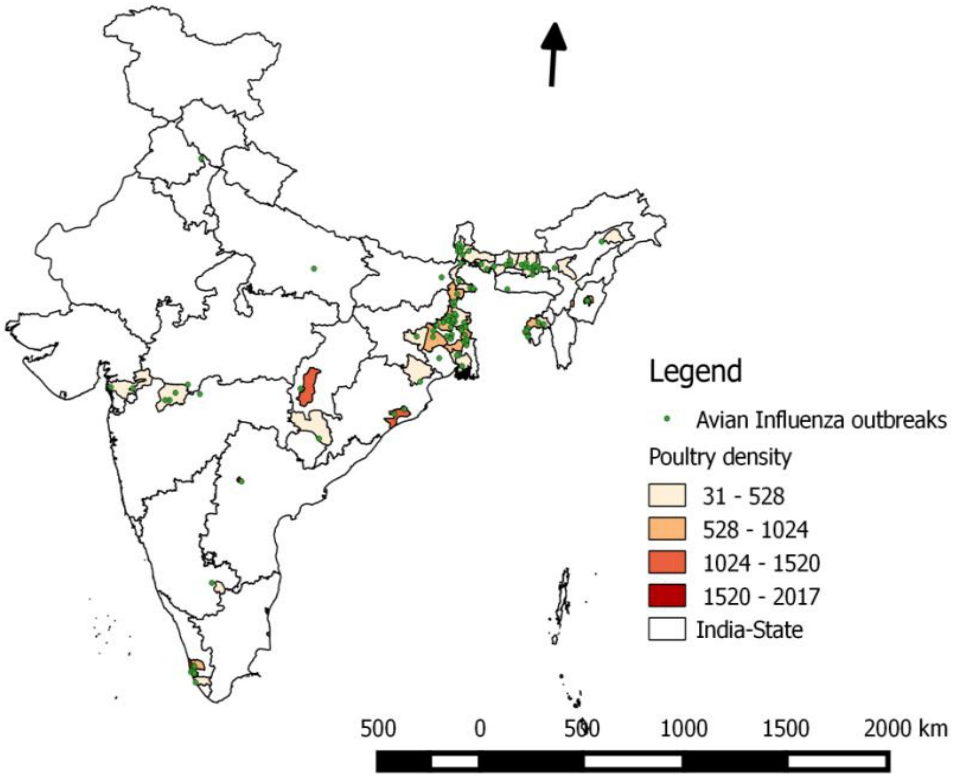


Fig 6: Spatial map depicting the poultry population density and avian influenza outbreaks in different districts from 2006-2015

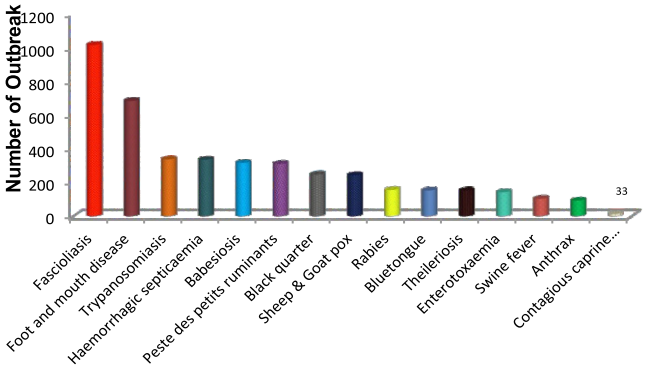
INSTITUTE SERVICE PROJECTS

IPC: ANSCNIVEDISIL201100100020 Project ID: IXX08329

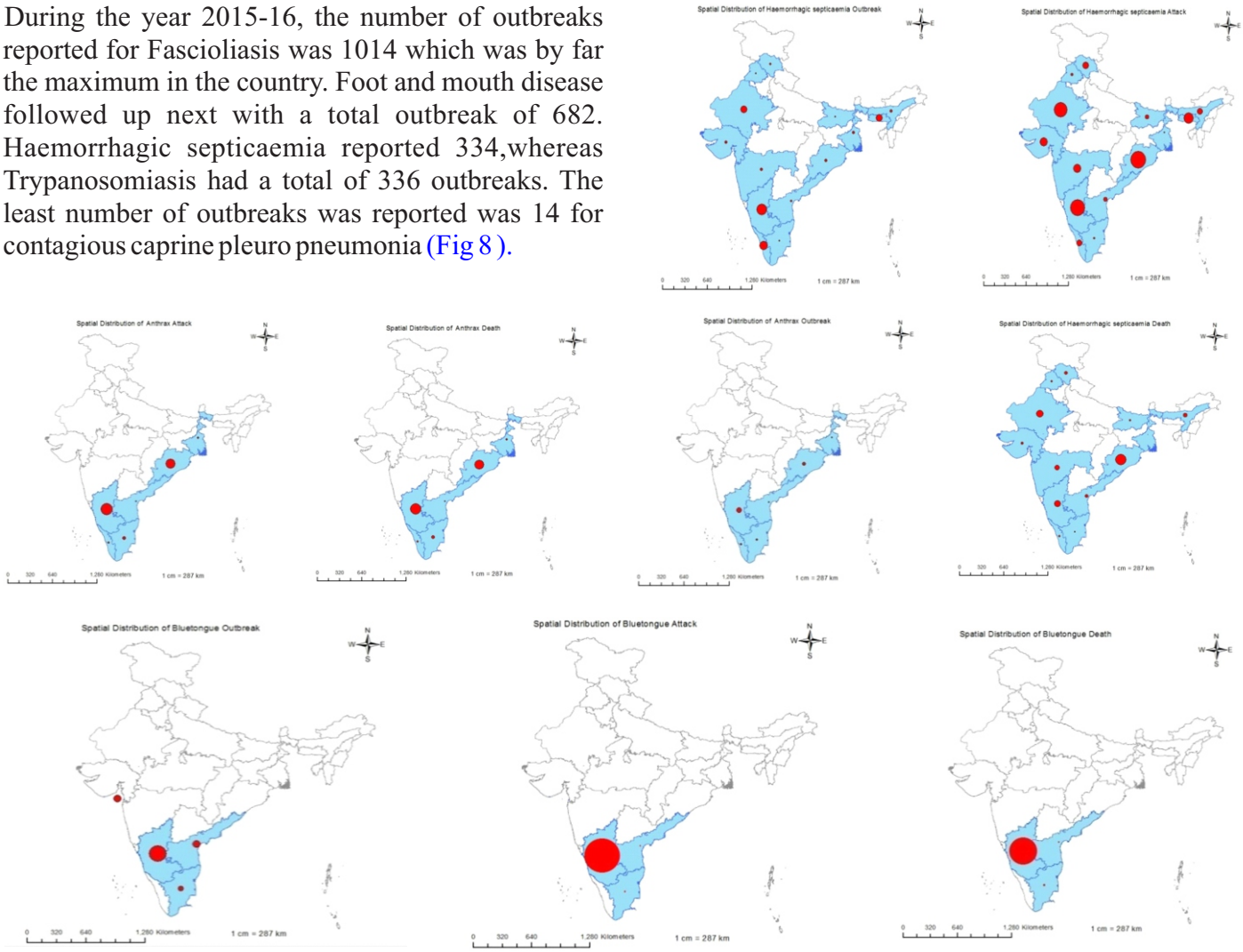
National Animal Disease Referral Expert System (NADRES)

D Hemadri, K P Suresh, S S Patil, G Govindaraj, Md. Mudassar Chanda,
J Hiremath and G B Manjunatha Reddy

The NADRES database contains information on major livestock diseases of the country along with their associated risk factors. Using these databases, the probability of occurrence of disease outbreaks at the district level was forecasted two months in advance. The results are available to any user on interactive basis at the website (www.nadres.res.in). The forecasts were also sent in as report to DADF and other directors of animal husbandry departments for taking up suitable preventive measures. Given below are examples of maps (Fig 7) generated using the disease information entered in the NADRES server.



During the year 2015-16, the number of outbreaks reported for Fascioliasis was 1014 which was by far the maximum in the country. Foot and mouth disease followed up next with a total outbreak of 682. Haemorrhagic septicaemia reported 334,whereas Trypanosomiasis had a total of 336 outbreaks. The least number of outbreaks was reported was 14 for contagious caprine pleuro pneumonia (Fig 8).



IPC:ANSCNIVEDISIL201100300022 Project ID: IXX08279

Maintenance and updating of National Livestock Serum Repository

D Hemadri, K P Suresh and S S Patil

As part of the sero-surveillance activity under AICRP on ADMAS, NIVEDI designs and sends sampling plan every year to each of the centers of AICRP on ADMAS. The serum samples so collected, as per the plan, are sent to NIVEDI for screening against various livestock diseases mainly, Brucellosis (Bovine, Caprine, Swine), Infectious Bovine Rhinotracheitis (IBR), Classical Swine Fever, Bluetongue, Leptospirosis etc. The serum bank at ICAR-NIVEDI, arranges for screening against the said diseases and catalogues the serum along with the results. During the year 2015-16, a total of 10753 serum samples were received from 23 centers of AICRP on ADMAS. The detailed statewise and specieswise distribution of the serum samples is given below (Fig 9a & 9b).

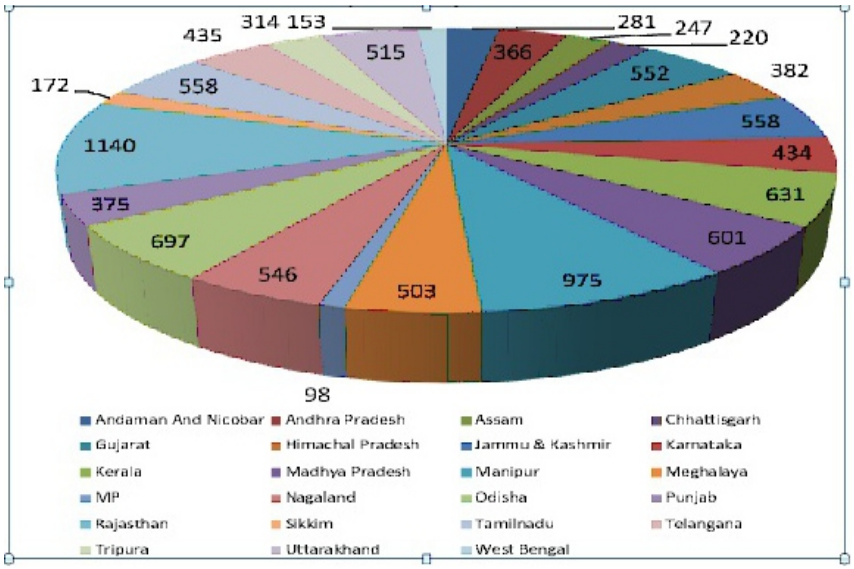


Fig 9a: Statewise distribution of serum samples received during 2015-16

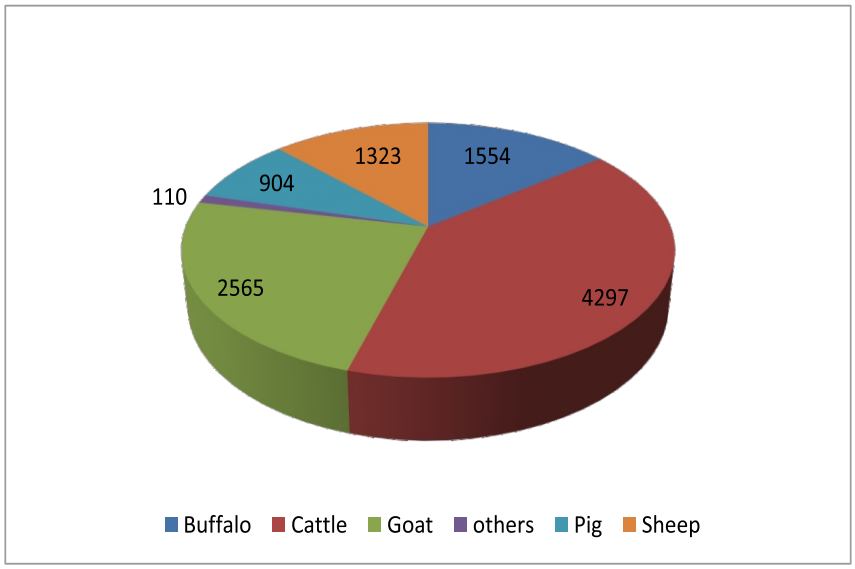


Fig 9b: Species wise distribution of serum samples received during 2015-16

IPC:ANSCNIVEDISIL201300200045 Project ID: IXX10708

Seroepidemiology of Bovine Brucellosis

R Shome, B RShome and M Nagalingam

During the period, a total of 7149 random sera samples [cattle (2421), buffalo (1293), sheep (1116), goat (2184) and swine (135)] received from 15 AICRP centres such as Andhra Pradesh (365), Chhattisgarh (220), Gujarat (538), Himachal Pradesh (382), Jammu & Kashmir (597), Kerala (530), Madhya Pradesh (1710), Meghalaya (262), Odisha (379), Punjab (375), Rajasthan (577), Sikkim (133), Telangana (435), Uttarkhand (501) and Assam (145) were screened for brucellosis by Protein-G iELISA kit, sheep & goat iELISA kit and laboratory standardized swine protocol for swine brucellosis. When state wise disease seroprevalence was compared, the highest seroprevalence was recorded in Telangana [57/435(13.10%)]; followed by Jammu &Kashmir [59/597 (9.88%)], Punjab [27/375 (7.20%)], Gujarat [31/538 (5.76%)], Rajasthan [31/577 (5.37%)], Andhra Pradesh [19/365 (5.20%)], Madhya Pradesh [25/1710 (1.46%)], Himachal Pradesh [5/382 (1.3%)] and Odisha [4/379 (1.05%)]. The other states such as Kerala, Uttarkhand and Chhattisgarh have shown <1% seroprevalence i.e[4/530 (0.75%)], [3/501 (0.59%)], [1/220 (0.45%)], respectively. Seroprevalence of brucellosis was not recorded in Assam, Meghalaya and Sikkim. Among livestock species screened (cattle, buffalo, sheep, goat and pig), highest seroprevalence was recorded in sheep 139/1116 (12.45%) followed by buffalo 51/1293 (3.94%), goat 38/2184 (1.73%), cattle 37/2421(1.52%) and lowest prevalence in pig samples 1/135 (0.74%). An overall prevalence of 3.72% (266/7149) was recorded in the random samples across the country. The state and species wise sample results are presented in Table 5 and Fig10

Table 5: Sero- prevalence of brucellosis in livestock species state wise in the year 2015-2016

Sl.no.	State	Cattle*	Buffalo*	Sheep**	Goat**	Pig***	Total	Percent Positivity
1	Andhra Pradesh	34(1)	128(4)	139(10)	56(4)	8(0)	365(19)	5.20%
2	Assam	145(0)	0	0	0	0	145(0)	0%
3	Chattisgarh	166(1)	20(0)	2(0)	32(0)	0	220(1)	0.45%
4	Gujarat	135(2)	171(9)	127(11)	105(9)	0	538(31)	5.76%
5	Himachal Pradesh	225(1)	38(0)	49(0)	70(4)	0	382(5)	1.30%
6	Jammu & Kashmir	146(0)	71(0)	171(54)	209(5)	0	597(59)	9.88%
7	Kerala	254(4)	22(0)	0	254(0)	0	530(4)	0.75%
8	Meghalaya	100(0)	0	0	123(0)	39(0)	262(0)	0
9	Madhya Pradesh	435(8)	296(9)	185(4)	726(4)	68(0)	1710(25)	1.46%
10	Odisha	174(4)	35(0)	63(0)	106(0)	1(0)	379(4)	1.05%
11	Punjab	101(11)	224(13)	23(1)	27(2)	0	375(27)	7.20%
12	Rajasthan	103(3)	146(12)	104(13)	215(3)	9(0)	577(31)	5.37%
13	Sikkim	79(0)	0	0	46(0)	8(0)	133(0)	0
14	Telangana	83(2)	53(4)	244(46)	53(4)	2(1)	435(57)	13.10%
15	Uttarakhand	241(0)	89(0)	9(0)	162(3)	0	501(3)	0.59%
	TOTAL	2421(37)	1293(51)	1116(139)	2184(38)	135(1)	7149(266)	
		1.52%	3.94%	12.45%	1.73%	0.74%	3.72%	

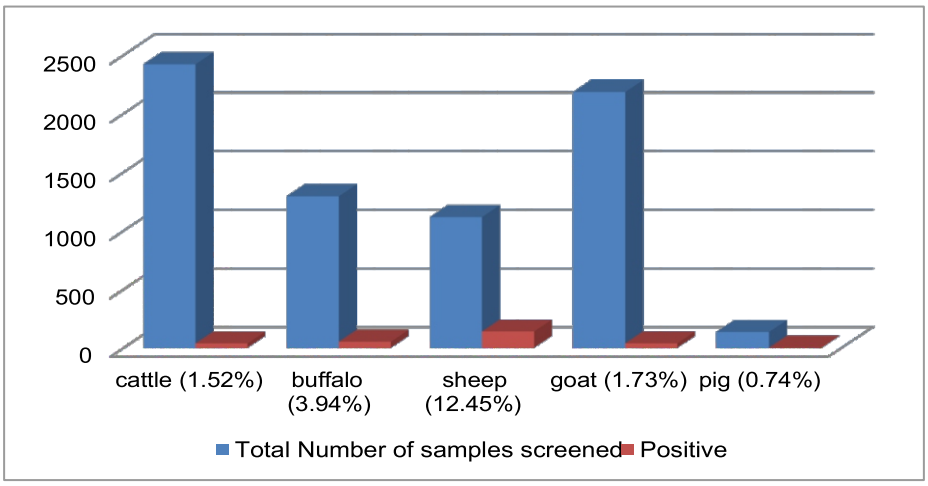


Fig 10: Species wise total number of samples screened in the year 2015-16

IPC:ANSCNIVEDISIL201200800032 Project ID: IXX10709

Seroepidemiology of Infectious Bovine Rhinotracheitis in India

S S Patil, D Hemadri and K P Suresh

IBR is an acute, contagious respiratory disease of cattle caused by bovine herpesvirus type 1 (BHV-1), commonly affecting the respiratory tract and the reproductive system. It is highly contagious, resulting in rapid spread of respiratory disease among cattle in close confinement, particularly in feedlots and when groups of cattle are transported. Disease outbreaks can result in severe production losses, abortion and mortality. India is endemic to IBR and vaccination is not practiced in our country. A total of 5883 sera samples from different states of India were tested, out of which 1224 samples were found to be positive for the presence of IBR antibodies using Avidin-Biotin ELISA. The highest prevalence rate of 50.54 was observed in Chattisgarh and the lowest prevalence was found to be 8.27 in Rajasthan (Table 6). The cumulative seroprevalence of IBR during 1995-2016 was 35.27%.

All the 16 virus isolates were revived and maintained in MDBK cells. A total of 11 IBR serums based Avidin Biotin-ELISA kits were supplied to different labs of the country.

Table 6: Sero-prevalence of IBR in different states of India

Sl no	State	Total no of sample	Positive sample	Percent positive
1	Andhra Pradesh	149	28	18.79
2	Assam	469	55	11.73
3	Chattisgarh	186	94	50.54
4	Gujarat	358	55	15.36
5	Himachal Pradesh	182	16	8.79
6	Jammu and Kashmir	427	133	31.15
7	Karnataka	315	106	33.65
8	Kerala	573	93	16.23
9	Madhya Pradesh	731	134	18.33
10	Manipur	412	102	24.76
11	Meghalaya	153	38	24.84
12	Orissa	363	86	23.69
13	Punjab	302	100	33.11
14	Rajasthan	653	54	8.27
15	Sikkim	71	14	19.72
16	Tamil Nadu	57	17	29.82
17	Tripura	259	62	23.94
18	Uttarakhand	133	28	21.05
19	West Bengal	90	9	10.00
	Total	**5883**	**1224**	**20.81**

EXTERNALLY FUNDED PROJECTS

Outreach Programme on Zoonotic Diseases

V Balamurugan, P P Sengupta and R Sridevi

The seropositivity of 38% (114/300) leptospirosis was observed in human samples collected from pyrexia of unknown origin (PUO) cases with prevalence of major of *Leptospira* serovars representing serogroups specific antibodies against Australis, Bankinang, Tarassovi, Ictero haemo- rrhagiae and Pomona, etc., when tested with 18 reference leptospira serovars in MAT (Table 7). On testing of 125 random purposive livestock serum samples from Gujarat, 46 samples were showed positive reactivity in MAT with 36.8% seropositivity. However, on testing of the 292 bovine serum samples with a history of fever, abortion and reproductive disorders, 231 samples showed positive reactivity in MAT, indicating the 79.10 % (Cattle, Buffaloes, Sheep and Goats) in 12 districts of Odisha during 2011–2014 tested for leptospirosis by MAT revealed the overall seroprevalence of 36.69% (197/537) with 36.13% in cattle, 54.28 % in buffaloes, 28.33% in goats and 44.44 % in sheep. All these, indicating the high percentage seropositivity of infection in humans and livestock, which warrants systematic one health approach study of epidemiology in order to know exact picture of the leptospirosis burden. Hence, the re-emerging nature of leptospirosis in India holds well and awareness about the disease has to be implemented in a proper way to safeguard the public health recording.

Table 7: Reference leptospira serovars used in Micro Agglutination Test

S.No	Species	Serovar	Strain	Serogroup
1.	*L. interrogans*	Australis	Ballico	Australis
2.	*L. interrogans*	Bankinang	Bankinang 1	Autumnalis
3.	*L. interrogans*	Canicola	HondUtrech IV	Canicola
4.	*L. interrogans*	Hardjo	Hardjo prajitno	Sejroe
5.	*L. interrogans*	Hebdomadis	Hebdomadis	Hebdomadis
6.	*L. interrogans*	Pyrogenes	Salinem	Pyrogenes
7.	*L. borgpetersenii*	Tarassovi	Perepelicin	Tarassovi
8.	*L. interrogans*	Icterohaemorrhagiae	RGA(ATCC443642)	Icterohaemorrhagiae
9.	*L. interrogans*	Pomona	Pomona	Pomona
10.	*L. Santarosai*	Shermani	1342 K	Shermani
11.	*L. inadai*	Kaup	LT 64 - 68	Tarassovi
12.	*L. kirschneri*	Grippotyphosa	MoskvaV	Grippotyphosa
13.	*L. fainei*	Hurstbridge	BUT 6	Hurstbridge
14.	*L. borgpetersenii*	Javanica	Poi	Javanica
15.	*L. noguchii*	Panama	CZ 214 K	Panama
16.	*L. interrogan*	Djasiman	Djasiman	Djasiman
17.	*L. interrogan*	Copenhageni	M 20	Icterohaemorrhagiae
18.	*L. interrogan*	Bataviae	Swart	Bataviae

Further, during the period under report, a total of 260 human serum samples were also screened for toxoplasmosis by using commercial diagnostic kit namely *Toxoplasma gondii* IgG and IgM ELISA kit (Toxoplasma IgG & IgM, DIESSE Diagnostica Senese, Italy Enzywell) as per manufacture's protocols. Out of these, 53 human serum samples with a case of PUO were showed positive reaction. The seropositivity of 20.38 % (53/260) and 2.69% (07/260) was observed against IgG- and IgM-toxoplasma specific antibodies, respectively. However, seropositivity of 15% (3 /20) was observed in human neuro toxoplasmosis cases.Further, seropositivity of 16.30 % (30/184) and 30.26 % (23/76) was observed in male and female, respectively with respect to toxoplasmosis.

All India Network Programme on Bluetongue (AINPBT)

D Hemadri and Md. Mudassar Chanda

As per the official reports there were 151 outbreaks of bluetongue involving 4 southern states of India namely Karnataka, Telangana, Andhra Pradesh and Tamil Nadu in that order (Fig 11). As evident in the figure large number of outbreaks (n=118) were recorded in Karnataka state involving 14 districts spreading across eight months starting from July 2015. In other states, the disease was recorded between December to February months.

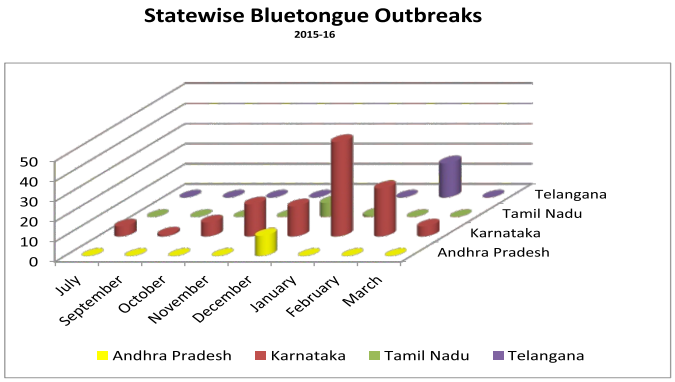


Fig. 11 Statewise bluetongue outbreaks (2015-16)

In addition, the suspected bluetongue outbreaks were investigated following unofficial reports (Fig12). Investigations of the outbreaks indicated that although sheep were mainly affected by the disease, involvement of goats was also noticed in few places. In addition, variation in severity of clinical disease and case fatality rates was also found. The most severe (based on clinical signs) disease was noticed in Koppal, Chikkaballapur and Tumkur districts.

Besides blood, clinical samples in the form of spleen from deceased animals were collected and after RNA isolation and reverse transcription, these were subjected to PCR using BTV OIE primers set 1. Nine of the 21 samples showed positive amplification. Ten blood samples received from Tamil Nadu were negative for BTV in PCR. Meta analysis of sero-prevalence studies on bluetongue was carried to evaluate the bluetongue prevalence in the country. Various epidemiological studies on bluetongue

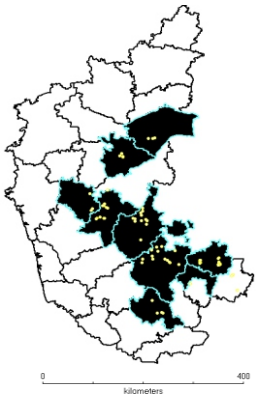


Fig. 12 Spatial locations of suspected bluetongue outbreak

seroprevalence published by different studies in different parts of India and also from disease reports submitted by respective states were collected. Studies reporting seroprevalence of BT for sheep, goats, cattle and buffalo were selected. The data from literature search was designed to carry out in two batches i,e 2005 to 2009 and 2010 to 2014 whereas, the states were grouped according to their regions. The estimated seroprevalence in sheep population was obtained from 55 studies with a sample size of 1,02,318. It had 58% (95% C.I: 31% -81%) prevalence in the southern states and the northern part of the country was 37% (95% C.I: 15%-66%) with a high I^2 value showing heterogeneity. The estimated seroprevalence in goat population were obtained from 44 studies with a sample size of 13,085 and a moderately high I^2 value showing heterogeneity. The prevalence was 52% (95% C.I: 20%- 82%) in southern states, while in the north it was calculated to be 30% (95% C.I 11%- 61%). The prevalence of cattle, buffalo and mithuns were grouped under 'others' category with the data obtained from 17 studies having a sample size of 4686.The prevalence was 73% (95% C.I: 0.1%- 1%) in southern states while in the North it was calculated to be 33% (95% C.I 10%- 99%).These figures may be useful in estimating the current trend of the Bluetongue in the country and for projecting the cost-benefits of preventive measures.

IPC: ANSCNIVEDICOP201500100064 Project ID: OXX02963

National Innovations on Climate Resilient Agriculture-Livestock disease surveillance in relation to weather data and emergence of new pathogens

B R Shome, P Krishnamoorthy, K P Suresh, G B Manjunathareddy, S S Patil, G Govindaraj, R Yogisharadhya and A Prajapati

A total of 337 Anthrax outbreak data in Karnataka state from the year 2000-2014 at village level collected from Department of Animal Husbandry, Karnataka. Subsequently data on latitude / longitude has been generated. Four remote sensing parameter were used for the estimation of climate factor of the Anthrax disease (NDVI, LST, NDWI, NDMI). Normalized Difference Vegetation Index (NDVI) is an index describing vegetation by showing the difference between near-infrared (which is strongly reflected by vegetation) and red light (which is absorbed by vegetation)and Land Surface Temperature (LST) can be defined as the temperature felt when the land surface is touched with the hands or it is the skin temperature of ground.

The Normalized Difference Water Index (NDWI) enhancing and accordingly measured using MODIS image and NDMI and NDWI were measured from Landsat8 image for village level data. We obtained overall average of 0.426 (NDVI), 33. 03 (LST), 0.28 (NDWI), 0.27 (NDMI). From the village level data, we calculated district level outbreak and value of each remote sensing parameters. The analysis we used Poisson regression model. Where the dependent variable is number of outbreak. The risk map of anthrax in state of Karnataka is depicted in Fig 13

Modelling the outbreak of Anthrax using climatic factors

Poisson Model log(Result)= 0.9033-0.1091(NDVI) -0.0140(LST) -0.0001(NDWI) +1.2369(NDMI)

This implies Result=exp{0.9033-0.1091(NDVI) -0.014 0(LST) -0.0001(NDWI) +1.2369(NDMI)}

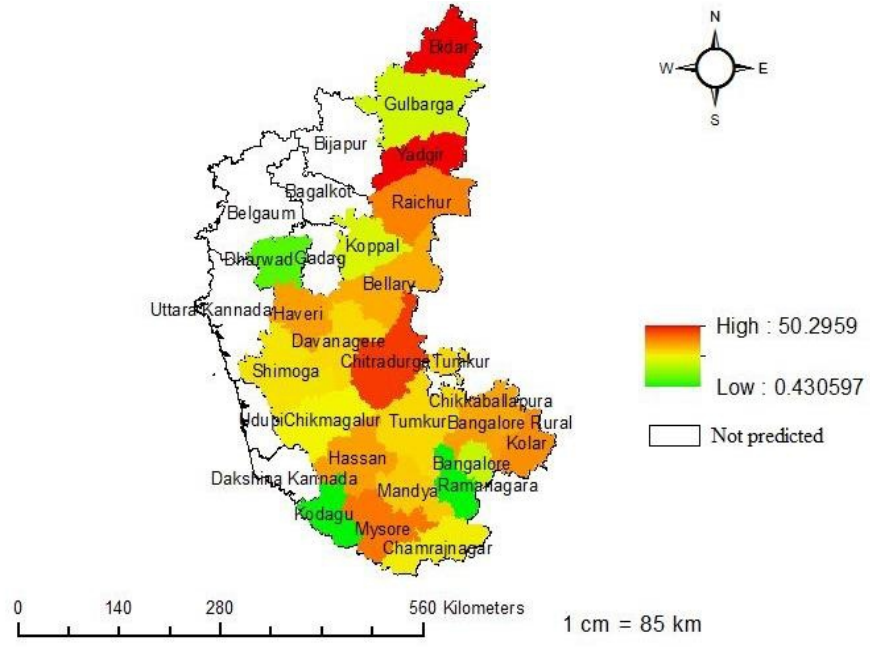


Fig 13: Risk map for Anthrax in Karnataka

IPC: ANSCNIVEDISOL201600200071

Evaluation of vaccine effectiveness and identification of the factors that affect field level vaccine efficacy of the vaccines against diseases under control program

J Hiremath, D Hemadri, R Shome, V Balamurugan, S S Patil, K P Suresh, Md. Mudassar Chanda and G B Manjunatha Reddy

Efficacious vaccines and effective vaccination are basis for any disease control programs. Hence evaluation of current vaccines for protective ability in field condition is critical. To achieve this project was formulated and submitted to ICAR-Extramural Funding of ICAR for XII plan period. The project got approved for funding with council letter void F. No. AS/8/20/2015-ASR-IV (Part-II) dated 31st March 2016. The research objectives of the project includes, evaluation of vaccine effectiveness of vaccines against FMD and PPR, identification of factors that affect the vaccine effectiveness of vaccines against FMD and PPR and identification of factors responsible for animal owners vaccine hesitancy. The project work has been initiated.

IPC: ANSCNIVEDISOL201600300072

Understanding the epidemiology of *Culicoides* Borne Diseases (CBD's) in wild and domestic ruminants

Md. Mudassar Chanda, D Hemadri, P P Sengupta, J Hiremath and S B Shivachandra

The proposed project aims to understand the role of different *Culicoides* species in transmission of orbiviruses in wild and domestic ruminants. The understanding will help to know the status of orbiviruses in wild ruminants and also their role in transmission to domestic livestock. This will help to adopt integrated control measures like vector abatement or vaccination. The project was approved on 31st March 2016 by ICAR. The project work has been initiated.

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

In this year, a double antibody sandwich ELISA (Ag detecting) was developed and the application for patenting of Ag-ELISA has been filed (Patent application no.3095/CHE/2015).A Total of 361 bovine samples from Karnataka (166), Odisha (91), West Bengal (104) were screened by ELISA. An overall 122 (33.8%) samples were found positive for the presence of antibodies of surra. The highest sero-positivity (S.P) was observed in Karnataka with 37.34%, followed by Odisha with 31.86%, and finally in West Bengal with 29.8% S.P. The sero-positive percentage in different state has been depicted in Fig14.

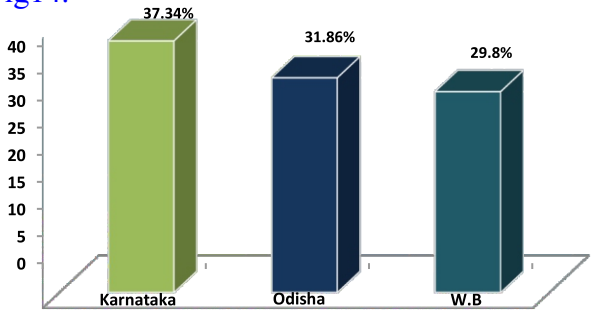


Fig14: Sero-prevelence of surra in bovines

IPC: ANSCNIVEDICOL201600100070 Project ID:OXX03486

External validation of diagnostic assays for detection of anti brucella antibodies developed under the DBT-network project on brucellosis

R Shome and M Nagalingam

The project funded by Department of Biotechnology (DBT) to perform third party validation for the developed diagnostic assays under the DBT-Network Project on Brucellosis. The project is multi-institutional and the collaborating partners are Translational Research Platform for Veterinary Biologicals, (TRPVB) Tamil Nadu Veterinary and Animal Sciences University, Chennai and ICAR - National Institute of Veterinary Epidemiology and Disease Informatics (ICAR-NIVEDI), Bengaluru.

The role of ICAR-NIVEDI and TRPVB in this project includes evaluation of the diagnostic kits that have been developed to detect anti- *Brucella* antibodies (second and third party validations), to facilitate TRPVB by providing standard sera from culture positive and negative animals, sera from experi--mentally vaccinated animals and field test sera samples for preparation of common panel for evaluation of diagnostic tests. This project has been initiated during February, 2016 and is for two years.

IPC: ANSCNIVEDICOL201201500039 Project ID: OXX02733

DBT Network Project on Brucellosis: Project Monitoring Unit (PMU)

H Rahman and G B Manjuntha Reddy

Brucellosis is an economically important and zoonotic disease in animals and human. The DBT-Network Project on Brucellosis is a multi-intuitional Pan-India programme. The project has different subunits on brucellosis epidemiology (8), Brucellosis diagnostics (3), Brucellosis vaccine (2), Brucellosis repository (1) and Brucellosis bioinformatics (1), with overall monitoring of project entrusted to Project Monitoring Unit (PMU) at ICAR-NIVEDI, Bengaluru. PMU is involved in co-ordinating different activities of all the subunits under DBT Network Project on Brucellosis. The multiplex PCR was developed by DFRL. Monitoring the research activities of different centres by means of monthly and quarterly reports, also submitting the compiled monthly, quarterly and annual reports to DBT. PMU coordinated Annual Review Meet at Mysuru incollaboration with DFRL, DRDO, Mysuru (21.11.2016). The regular updating and maintenance of DBT-Brucellosis website is done at MKU. PMU organized DBT sponsored "Interactive workshop cum midterm review meet of DBT-Network Project on Brucellosis" on 4th July, 2015 at NIVEDI, Bengaluru and coordinated Brucella genomics workshop at MKU, Madurai for the PI/CoPIs/contractual staff working under different subunits of network project. PMU co-ordinated in sending the serum samples, bacterial cultures, *Brucella* antigens procurement, DNA between the different subunits. PMU also coordinated the validation of different Brucella diagnostic kits developed under the project and multiplex PCR developed by DFRL.

IPC: ANSCNIVEDISOP201201600040 Project ID:OXX02578

DBT Network project: Brucellosis Epidemiology (BE-1)

R Shome, B R Shome and M Nagalingam

A total of 671 serum and blood samples from various livestock species were evaluated by PCR and qPCRs. The PCR shown 0.69 and 0.79 kappa agreement for blood and serum DNA samples and 0.8 and 0.9 kappa agreement for DNA from blood and serum samples by qPCR indicating the superiority of real time PCR over conventional PCR in detecting the *Brucella* DNA in blood/serum samples.

Brucellosis outbreak investigation in 2 pig farms with a total of 492 (Farm 1, n=357; Farm 2, n=135) serum samples, revealed 75% samples from farm1 and 39% from farm 2 were positive for antibrucella antibodies by RBPT and iELISA which showed the increased prevalence of the porcine brucellosis and lack of awareness about the disease among the pig owners. Investigation of brucellosis among high risk group individuals in organised dairy farms (n- 464) in Karnataka were screened for brucellosis which revealed a high seropositivity of 17.46%.

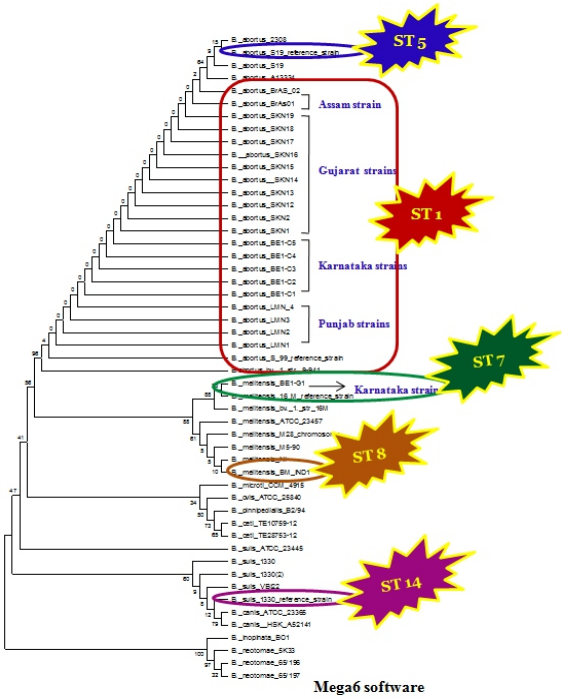


Fig 16: Phylogenetic analysis of MLST sequences of *Brucella* sp.

High seropositivity was recorded among the veterinarians (30.23%), followed by paravets technicians (26.92%) and livestock inspectors (21.79%). Risk factors like contact with animals, assisting parturition, handling aborted material, removal of placental material and raw milk consumption were found to be significantly associated with the disease. Symptoms like myalgia, joint pain and combined symptoms of myalgia and joint pain were significantly more among sero - positives. MLST typing of 10 more *B. abortus* isolates to the already 26 sequence types were analysed and in MLST typing, field *Brucella* isolates showed the close genetic similarity of the *Brucella* vaccine strain *B. abortus* S19 and antigenic strain *B. abortus* S99. In NCBI Gen bank database, 53 draft genome seqauences of *B. abortus* field isolates from different hosts and countries during the period of 2000-2014 were analysed and compared to Indian sequence types (n-36). Among 53 STs, 40 were typed as ST1 indicating the predominance of ST1 among the *B. abortus* genotypes affecting the livestock in India and elsewhere in the world (Fig16).

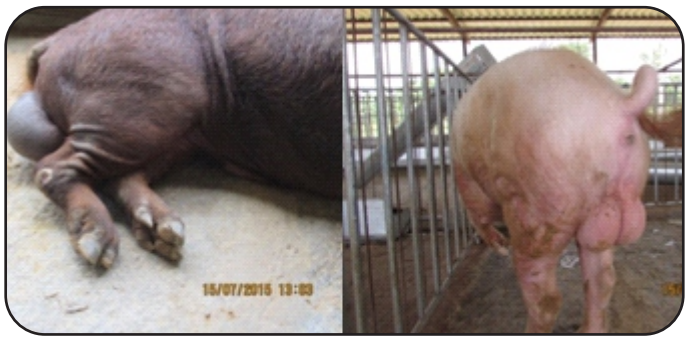


Fig 15: Orchitis in *Brucella* infected boar

IPC: ANSCNIVEDISOP201201700041 Project ID: OXX02384

DBT-Network Project: Brucellosis Diagnostics (BD-2)

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

The target genes which were identified (bls, bp26, sod c, p39) for the production of either individual and/or multiple recombinant proteins that can be used in diagnostics were amplified from *Brucella abortus* S99, cloned in pET32(a) vector. *E.coli* BL21 (DE3 pLysS)cells were transformed with recombinant plasmids bp26 and bls. Expression was carried out by induction with IPTG using standard conditions and analyzed by SDS-PAGE and western blot. Optimization of expression of these cloned genes in *E.coli* and its characterization were carried out. During the period under report, recombinant BP26 and L7/L12 proteins were received from BV-1 Jawaharlal Nehru University Centre, New Delhi and their suitability in using them as a diagnostic antigen were tested and observed that BP26 may be further explored to use as diagnostic antigen whereas L7/L12 protein may not be a suitable candidate for diagnostic antigen. Expression of sodC and p39 proteins was carried out and optimized expression of sodC protein. In addition bulk production of BP26 and BLS were carried out. Standardization of rBP26 and BLS based ELISA using limited number of field serum samples was carried out. Sensitivity and specificity and other statistics were calculated. Further standardization of recombinant antigen based ELISA either as single protein or together and development of latex agglutination test (Fig 17) using rBP26 is in progress for the diagnosis of bovine brucellosis.

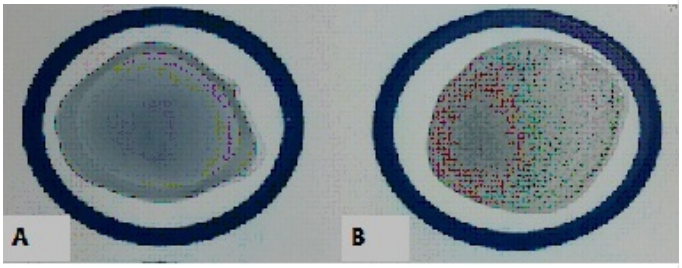


Fig 17: Latex agglutination test using rBP26 antigen in bovine negative (A) and positive (B) serum

IPC:ANSCNIVEDISOL201400200055 Project ID: IXX03176

DBT- Programme for NER on Advanced Animal Disease Diagnosis and Managament Consortium (ADMaC)

Sub_Project 1: Sero-Epidemiology study of Brucellosis in livestock in North East states of India using ELISA and Fluorescent Polarization Assay

R Shome, R Sridevi and G B Manjunathareddy

Fluorescence Polarization Assay (FPA) is capable of distinguishing vaccinal antibody and antibody due to infection was standardized using O-Polysaccharide (OPS) extracted from *B. abortus* S99 conjugated to a Fluorescence Isothiocyanate (FITC), purified by DEAE anion exchange chromatography and was utilized for the downstream process for assay development as a tracer. The tracer concentration with total fluorescence intensity value in the range of 250000-500000, serum dilution of 1:50 and cutoff value of ≥25 delta mP were considered optimal concentrations. Panel of 400 bovine serum samples (positive=60, Negative=240) and *B. abortus* S19 calfhood vaccinated=100] selected based on RBPT and iELISA test results were screened by FPA. In ROC curve using MedCalc 15.8 software has revealed 82.64% of area under the curve, with 88.89% and 93.75% of relative sensitivity (SE) and specificity (SP) respectively for cattle. Similarly, a panel of 100 each serum samples from small ruminants and pigs (positive=20 and negative=80) were tested and AUC was found to be 0.857 and 0.841 and SE and SP of 75% and 97.5%, and 75% and 98.75%, were recorded for small ruminants and swine, respectively (Fig). The FPA has been evaluated for DIVA potential using 497 *B. abortus* S19 calfhood vaccinated serum samples collected on 0^{th}, 14^{th}, 28^{th}, 45^{th}, 60^{th}, 90^{th}, 120^{th} and 150^{th} DPV (days of post vaccination) (cattle=52 and buffalo=19 at eight different collection intervals).

The samples were tested by RBPT, SAT, iELISA, and FPA. The animals till 150 DPV were found positive by RBPT, iELISA and SAT and nearly all animals were found negative by FPA. This clearly indicated that the indigenously developed FPA test can be used from 21 days post vaccinated animals for differentiation of vaccinial versus infection antibodies.

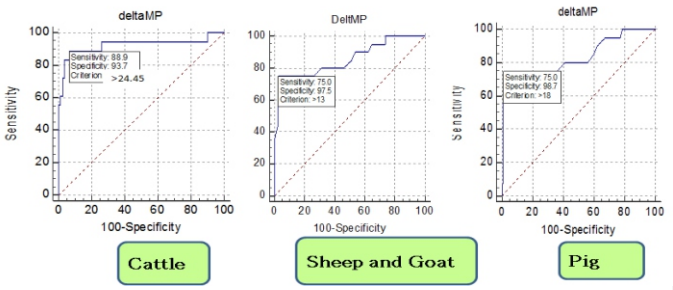


Fig 18: ROC for evaluation of Fluorescent Polarization Assay

IPC:ANSCNIVEDISOL201400300056

Project ID:OXX03175

Sub-Project 2: Epidemiology study of Classical Swine Fever virus (CSF), Porcine Reproductive and Respiratory Syndrome virus (PRRSV) &Tarque TenoVirus (TTV) from North East (NE) region of India

D Hemadri, S S Patil, V Balamurugan, G B Manjunatha Reddy, J Hiremath and G S Desai

A total of 382 samples (Blood=4, Serum=355 and Tissues=23) from different states of North-East region were tested using commercial enzyme-linked immunosorbent assay (ELISA) kits for serum samples and for blood and tissue using Qiagen RT PCR(reverse transcriptase polymerase chain reaction) kit. The mean percent positivity of CSFV in suspected samples was 66.49% (254/382). State wise percent positivity for CSFV is Assam 62.8% (66/105), Mizoram74.5 % (44/59), Meghalaya 73.9% (142/192), Arunachal Pradesh 77.7% (7/9), Sikkim (both negative), Tripura (both negative). This baseline data shows the probable high prevalence of CSFV in the north east region.

A total of (49) Blood, (37) Tissue and (141) Serum samples from pigs of different states of India were screened for the presence of both TTSuV 1&2 by conventional PCR method targeting 260bp of non-coding region. 21 serum samples from Mizoram, 12 from Manipur and 7 from Arunachal Pradesh were screened against both TTSuV1&2, out of which 2 serum samples from the state Mizoram were found positive for TTSuV2 and the rest were negative.

Twelve tissue samples received from Assam were screened against both TTSuV1&2 and found negative. A total of (4) blood samples received from Mizoram were also screened for the presence of both TTSuV1&2 and found negative.

A total of 311 serum samples received/collected from April 2015 to March 2016 from different states of NE regions were tested using commercially available PRRSV Ab detection kit. State wise percent positivity for PRRSV was; Assam 1.63% (1/61), Arunachal Pradesh 0% (0/7), Madhya Pradesh 0.64% (1/154), Manipur 0% (0/12) and Mizoram 47.71% (32/67). This base line data shows the high prevalence of PRRSV in Mizoram compared to all the other states of North Eastern region. Twenty eight tissue samples collected from 11 different animals from Assam (8), Meghalaya (1) and Arunachal Pradesh (2) screened from PRRSV by PCR using Qiagen tissue RNA isolation, c-DNA synthesis carried out by Thermo-Scientific M-MuLV RT enzyme followed by PCR using NEB Taq Polymerase. Out of which, 3 tissue samples of same animal from Meghalaya was found positive. Similarly, 3 of the 4 blood samples from Mizoram were positive for PRRS.

Sub-Project 3: Development of Infectious Disease Information System (IDIS) and Risk assessment models for Trans-boundary animal diseases (TAD) & other emerging livestock diseases in NE Region of India

K P Suresh, G Govindaraj and S S Patil

Risk map prediction for CSF in North Eastern regions was attempted using the remote sensing applications and GIS. For this purpose, data viz. Land Surface Temperature (LST), and Normalized Difference Vegetation Index (NDVI), Distance from major cities (km), Distance from Highways (km), Distance from Roads (km), Distance from Railways (km), Distance from Water bodies (km), Rainfall were measured using earth observatory satellite images or maps. Using ArcGIS tool the raster layers are generated for each risk factor mentioned above. The Logistic regression model was employed for the risk map generation. The high risk of disease occurrence was predicted in Baksa, Darrang, Kamrup Metropolitan and Kamrup districts of Assam with a probability of occurrence of 0.6643. A moderate to low risk was predicted in the other areas of North East Regions with a probability of occurrence of around 0.3578. A very low risk of disease occurrence was predicted in predicted in the other areas of North East Regions with a probability of occurrence of around 0.3578. A very low risk of disease occurrence was predicted in Dibrugarh district of Assam and Phek district of Nagaland with a probability of occurrence of 0.0514. To conclude from the study, the risk maps can act as important epidemiological tools to measure the probability of occurrence of CSF outbreaks and may be used for risk prediction of any other diseases. The Predicted risk map for CSF incidence in North eastern region is presented in Fig 19

$$Y = \frac{1}{\left(1 + e^{-(-0.088 - 0.002 * \text{Distance from cities} + 0.0001 * \text{Distance from Highways} \ldots\ldots\ldots\ldots 1.432 * \text{NDVI})}\right)}$$

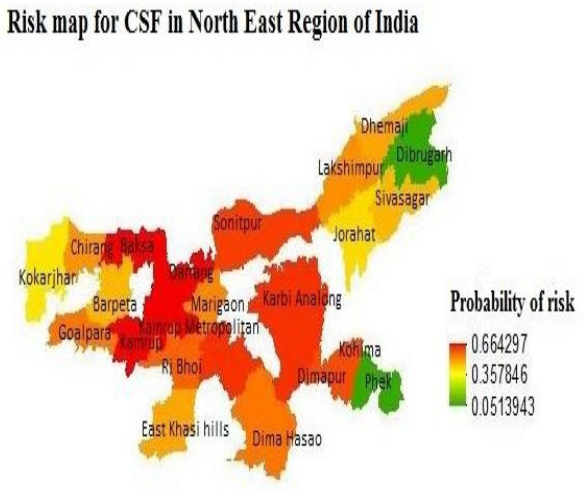


Fig 19: Risk map depicting the probability of occurrence of CSF outbreaks in North East Region of India

Sub-Project 4: Surveillance and Molecular analysis of MRSA, MR-CoNS, VRE, ESBL, & Carbapenemase producing gram negative bacteria in Farm animals and the animal handlers in North East Region in India

B R Shome, K P Suresh and P Krishnamoorthy

A preliminary random investigation of antibiotic resistance in livestock (cattle, pig, sheep, goat) and poultry carried out covering parts of Meghalaya and Assam found 41% (32/78) of *E. coli* to be ESBL/*Amp*C/MβL producer with CTXM-IV detected as the most common determinant across cattle, pig and poultry. Further testing these 32 resistant *E. coli*, noted occurrence of 9 (B/O, FIC, A/C, Y, I1, HI1, N,L/M,HI2) plasmid replicons types in 65.62% *E. coli* isolates. Integron Class 2 and 3 was found in *E. coli* of poultry and cattle. Among the isolates, 18.75% *E. coli* were found harboring Shiga toxin stx2 gene, originating from cattle faecal samples. Finally MLST detected 7 different Sequence Types (ST) with ST-10 a common clonal strain of *E. coli* in cattle and pig originating from distant geographical areas. ST1079 detected in Goat and Pig of two different regions. Comprehensive profiles of molecular character-istics revealed 28 *E. coli* genotypes circulating in livestock and poultry (Table 8). Importantly, a key observation of global epidemiological concern was the detection of a pig origin ST-10-MβL producing *E. coli* positive for multiple plasmid FIC, IncA/C, HI1 and HI2 and ST-617–*Amp*C, CTXM-1 producing *E. coli* positive for *Inc*A/C, *Inc*L/M, Integron class 2 and 3 (Table 8). The study demonstrates the animals as primary reservoirs.

Table 8: Molecular characterization of *E.coli* isolates circulating in livestock in poultry

Species	Location	Sample ID	Virulence	*ESBL*	*MβL*	*AmpC (N=4)*	*Plasmid (N=18)*	*Integron (Int)*	*MLST*
CATTLE	Farm 1	NE-C-2	*traT*	ctxmIV	-	-	Y	-	ST1727
		NE-C-4#		ctxmIV	-	-	-	-	
		NE-C-5	*fliC, traT*	ctxmIV	-	-	Y	-	
		NE-C-8	*stx2, fliC,*	ctxmIV	-	-	-	-	
	Farm 2	NEC-28#		ctxmIV	-	-	-	-	
		NE-C-29	*stx2*	ctxmIV	-	-	-	-	
		NE-C-33	*cnf1, traT*	-	-	*AmpC*	L/M	-	
		NE-C-34	*traT*	ctxmIV	-	-	-	-	
		NE-C-35	*stx2, traT*	ctxmIV	-	-	-	-	ST58
		NE-C-37	*stx2, traT*	ctxmI	-	-	HI1	-	
		NE-C-38	*stx2, traT*	ctxmI	-	-	-	-	
		NE-C-39	*stx2, traT*	ctxmI, tem	-	-	L/M	Int-2	**ST10**
	Farm 3	NE-C-53	-	ctxmI, tem	-	-	N	Int-2	**ST10**
PIG	Farm 4	NE-PG-20	-	Ctxm4	-	-	Y, L/M	-	ST1079
		NE-PG-24	*traT*	Ctxm4	-	-	L/M	-	
	Farm 5	NE-PG-59	*flicC, traT*	-	MβL	-	**IncA**/C	-	ST206
		NE-PG-60	*traT*	Ctxm1	-	-	-	-	
		NE-PG-69*	*traT*	Ctxm1	-	-	Y	-	
		NE-PG-70*	*traT*	Ctxm1	-	-	Y	-	
		NE-PG-71	*traT*	Ctxm1	-	-	L/M	-	
		NE-PG-72*	*traT*	Ctxm1	-	-	Y	-	
		NE-PG-74	*traT*	**Ctxm1**	-	***AmpC***	**IncA/C, L/M**	**Int-2, Int-3**	ST617
	Farm 6	**NE-PG-80**	*traT*	-	MβL	-	**FIC, Inc A/C, HI1, HI2**	-	**ST10**
POULTRY	Farm 7	NE-P-42	*traT*	Ctxm4	-	-	Y, L/M	-	ST746
		NEP-43#	-	Ctxm4	-	-	-	-	
		NEP-44	*traT*	Ctxm4, tem	-	-	-	-	
		NEP-45	*traT*	Ctxm4	-	-	B/O	-	
		NE-P-46	*traT*	-	-	*AmpC*	Y, B/O	Int-3	
		NE-P-48	*traT*	-	-	*AmpC*	Y, L/M,B/O	Int-3	
		NE-P49	*traT*	-	-	*AmpC*	B/O,HI2	Int-3	
		NE-P50	*traT*	-	-	*AmpC*	N,Y	-	
GOAT	Farm 8	NE-G-89	*traT*	-	-	*AmpC*	I1,HI2	-	ST1079

of multi drug resistant *E. coli* geno types contributing to the growing crisis of antibiotic resistance. Further, a total of 22.9% isolates were found to be resistant to three or more antimicrobials with highest occurrence of resistance noted for Ampicillin, Cefoxitin and third generation cephalosporin viz., Ceftriazone. The most common ARGs was bla_{TEM}(20.8%) detected acoss pig, cattle, sheep, goat and poultry, *Amp*C (6.25%) and Metallo β lactamase (MβL) (4.16%) in pig, followed by integrons in 4.16% and plasmids in 8.33 % isolates. Importantly two multi drug resistant *Klebsiella* isolates from Goat and two from Pig were found to carry promiscuous resistance plasmids replicon *Inc*I1 and Y respectively. Whole Genome Sequencing using Illumina Platform was performed for one representative multidrug resistant *E. coli* of poultry origin designated NIVEDI NEP44. WGS data analyses revealed the complete repertoire of virulence, bacteriocin and multidrug resistant Efflux Pumps in the emerging prevalent clone of MDR *E. coli* detected in the livestock providing deeper insights of the pathogen biology as depicted in Fig 20. This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession LUYD00000000.

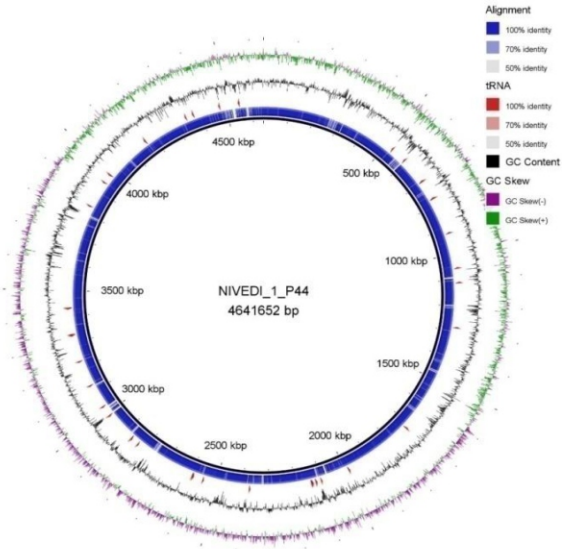


Fig 20: *Escherichia coli* NIVEDI NEP44 ring representation using BRIG 0.95. The contigs from NEP44 were ordered to match genomic arrangement using progressive Mauve. The inner ring shows the percent identity comparing with NEP44. The next ring alternating blue and red sections shows the contig delimitations for NEP44. The next ring shows the location of tRNAs according to Prokka v1.7 (Seemann, 2014). The last two (outer) rings show the GC content and skew.

IPC: ANSCNIVEDICOP2012019 00043

Project ID:OXX02254

Sero surveillance 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

The objective of the project is to study prevalence and viral genotype of PPRV in sheep and goats of north eastern states of India. The phylogenetic analysis of the N and F gene sequences of PPRV was amplified from the suspected clinical goat samples (n=12) revealed circulation of lineage IV virus in NE regions as like virus circulation in rest of India. The seropositivity of the PPR in goats using random samples collected from NE regions at various time periods showed, increased seropositivity of goats to PPRV {In 2012-2013 - 11.63% (37/318); 2014-2015- 18.31 % (37/202) and 2015-2016- 40.39 % (61/151)} The details of goat samples screened and its test results for the year 2015-2016 are shown in Table 9.

The seropositivity of the PPR in goats was 40.39%(61/151), when screened using random samples, whereas the seropositivity of 88% (22/25) was observed when tested suspected samples.

Table 9: Serodiagnosis of PPR

State	No. of samples tested	PPR CompetitiveELISA	
		Positive	Percent Positivity
Assam	85	80	94.11
Manipur	37	3	8.10
Tripura	54	3	5.55
Total	176	86	48.86

IPC: ANSCNIVEDICOP201300500048 Project ID: OXX02582

Sero-serveillance, molecular characterization and epidemiology of pox viral infections in animals from north eastern region of India

G B Manjunatha Reddy and V Balamurugan

The latest census data on total livestock population data covering different species of animals from North-Eastern States has been collected from Department of Animal Husbandry, Dairying and Fisheries for study of epidemiology of pox viral diseases and base line data has been created. The geo-coordinates were extracted prepared and GIS based mapping of livestock population for NER states for cattle, buffaloes, sheep and goats was prepared (Fig 21). The disease outbreaks data was collected and mapped for knowing the distribution pattern of outbreaks at district level. Standard vaccine strain of sheep pox virus was procured from ICAR-IVRI and is being maintained for further use in research as positive control both in vero cells and ovine testis epithelial cell line. The VNT was standardised for detection of anti-capripox virus antibodies and the serum samples received from main centre are being processed. The disease risk factors viz temperature, humidity, rain fall etc. are being complied for NER states for disease mapping. The livestock distribution maps for different species like cattle, buffaloes, sheep, goat, mithun and yak were prepared using ArcGIS. The designed questionnaire for collection of disease outbreak data for further epidemiological analysis has been sent to main centre for necessary use during field disease outbreak investigations.

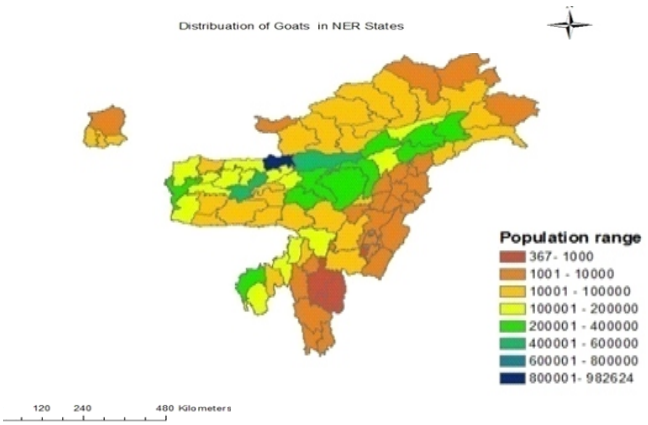


Fig 21: Distribution of goats in NER states

IPC: ANSCNIVEDICOP201300600049 Project ID:OXX02583

Prevalence and molecular epidemiology of BVD in ruminants with special reference to Mithun (*Bos frontalis*) in North Eastern States of India

D Hemadri and S S Patil

A total number of 1194 serum, 595 blood and 45 tissue samples collected from mithun, cattle, buffaloes, sheep and goats in the North Eastern states (Nagaland, Manipur, Mizoram and Arunachal Pradesh) and 14 tissue samples from pigs in Assam state were processed for determining the extent of BVDV infection. For determining prevalence of BVDV antibodies, virus neutralization test was used while for detection of BVD virus, real-time RT-PCR or virus isolation methods were employed. Of the 1194 sera samples tested by VNT, 19 animals (cattle-16; goat-3) from the states of Nagaland and Manipur were found positive for BVDV antibodies. Cross neutralization tests revealed prevalence of both BVDV-1 and BVDV-2 infection in cattle whereas BVDV-1 infection in goats. In contrast, all the leukocytes (595) and tissues (45) tested were found negative for BVDV upon testing by real-time RT-PCR or virus isolation. Tissue samples obtained from pigs (14) in Assam states suspected for CSFV and BVDV co-infection were found negative for BVDV-1 and BVDV-2 but were found positive for CSFV. It is evident from the test results that in contrast to high prevalence of BVDV infection in the mainland, there is low prevalence of BVDV infection in the NE region and there is no serological or virological evidence of BVDV infection in the sampled mithuns (*Bos frontalis*) from Nagaland, Manipur, Mizoram and Arunachal Pradesh. However, sampling and testing of more number of mithuns and other ruminants in this region will provide better insights into the BVD epidemiology.

IPC: ANSCNIVEDICOP201300700050 Project ID: OXX02584

Aetio-Pathology and molecular epidemiology of bacterial and viral diseases associated with the respiratory problems of yak in the North-Eastern states of India

R Shome, S S Patil and G B Manjunatha Reddy

A total of 40 and 160 yak and sheep, nasal samples respectively collected from respiratory infections were processed for isolation and four *M. haemolytica* isolates were recovered, identified by biochemical tests and confirmed by genus and species specific PCRs. From the same set of samples, 7 *P. multocida* type 'A' cultures were isolated and identified by biochemical tests and confirmed by *P. Multocida* species and *P. multocida* type 'A'type specific PCR [*P. multocida* (460bp) and Type 'A' (564bp)]. Among bacterial identifications, 16S rRNA sequencing has proven to be a stable and specific molecular marker for the identification of bacterial species. Partial 16S rRNA sequences of four *M. haemolytica* and six *P. multocida* type 'A' isolates were amplified, sequenced, analyzed and are available in NCBI (Accession NoKU517779, KU598685, KU598686 &KU598687, KU517780, KU533793, KU533779, KU585899, KU585900 & KU585901). Among the 4 isolates sequenced, sequences of 3 isolates were falling in separate cluster while the other in the same cluster along with the global isolates. Whereas all the six *P. multocida* type A isolates sequences were grouped in the same cluster with the other global isolates. The rapid direct speciation of *P. multocida* serotype 'A'&'B' and *M. haemolytica* species in clinical samples by multiplex PCR (mPCRs) was standardized initially by enriching nasal swabs in brain heart infusion (BHI) broth for 18hrs. The extracted DNA from enriched broth was used for mPCR by a panel of primers for detection of 2 species[*P. multocida*(460bp) and *M. haemolytica* (143bp)] & 2 serotypes of *P. multocida* [*P. multocida* Type 'B' (620bp) and Type 'A' (564bp)]. Among 160 sheep nasal samples processed, 88 (55%) and 13 (8.1%) samples showed amplification for *M. haemolytica* and *P. multocida*, respectively. Interestingly, all the 40 yak nasal samples were negative for *M. haemolytica* and *P. multocida* by mPCR. The study highlights importance of direct detection and speciation of *M. Haemolytica* and *P. multocida* type 'A' and 'B' in clinical samples for species and serotype based documentation of the disease in the country (Fig 22).

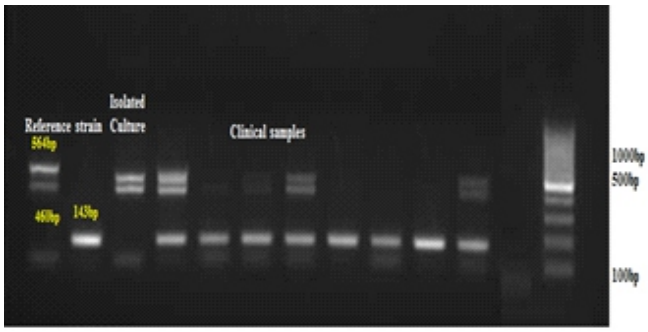


Fig 22: Evaluation of mPCR for detection of *P.multocida* species & type 'A' & 'B' and *M. Haemolytica* in clinical samples

Fig 23: Phylogentic analyses of *(a) M. haemolytica (b) P. multocida*