TRAINING PROGRAMME ON BASIC EPIDEMIOLOGY, PATHOLOGY AND KEY STEPS TO SETUP FIELD DIAGNOSTIC LABORATORY

Training Manual

25th – 27th September 2014

Advanced Animal Disease Diagnosis and Service Management Centres (AADSMC)
North Eastern Region Core Lab I
Assam Agricultural University
Khanapara Campus
Guwahati-781 022

TRAINING PROGRAMME ON BASIC EPIDEMIOLOGY, PATHOLOGY AND KEY STEPS TO SETUP FIELD DIAGNOSTIC LABORATORY

Training Manual

Compiled By
Dr N N Barman

Published by
AADSMC
North Eastern Region Core Lab I
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Training Schedule

Date	Time	Topic	Resource Person
25.09.2014 Thursday	9.00am-10.00am	Registration of the participants	
	10.00am-11.0am	Inauguration and Brief presentation on Project mandates	Dr. S. K. Das
	11.0am-11.30am	Group Photo and High Tea	
	11.30am-12.15pm	Animal Disease Monitoring and Surveillance	Dr. N. N. Barman
	12.15 pm-1.00pm	Principles of field epidemiology and collection of epidemiological data & their interpretation	Dr. Arnab Sen
	1.00pm-2.30pm	Launch	
	2.30pm-4.30pm	Group Activity : Design of a Microbiology laboratory and creation of facilities	Dept of Microbiology.
26.09.2014 Friday	9.00am-9.45am	Application of Geoinformatics in Epidemiology and Control of Animal Diseases	Dr.ArnabSen
	9.45am-11.00am	Post-mortem examination as an aid in disease diagnosis under field condition. Collection, dispatch and laboratory entry of samples.	Dept of Pathology., CVSc, AAU
	11.00am-1.00pm	Group Activity : Design of a Pathology laboratory and creation of facilities	Dept of Pathology
	1.00pm-2.30pm	Lunch	
	2.30pm-4.30pm	Group Activity : Design of a Pathology laboratory and creation of facilities	Dept of Pathology.
27.09.2014 Saturday	9.00am-9.45am	Laboratory support in investigation of an outbreak	Dr. T. K. Dutta
	9.45am-10.30am	An approach for diagnosis of Parasitic diseases	Dept of Parasitology, CVSc, AAU
	10.30am-1.00pm	Group Activity : Design of a Parasitology laboratory and creation of facilities	Dept of Parasitology.
	1.00pm-2.30pm	Lunch	
	2.30pm-3.00pm	Group discussion & feed back	All the PIs of 3 core labs including Director of Research, AAU
	3.00pm-4.30pm	Valedictory function	

Content

Proposal on a Tripartite Collaboration

ADVANCED ANIMAL DISEASE DIAGNOSIS AND SERVICE MANAGEMENT CENTERS (AADSMC)

Title in Sanctioned letter : *Advanced Animal Diagnostics and Services on Animal Health and Diseases* (ADSAHD)

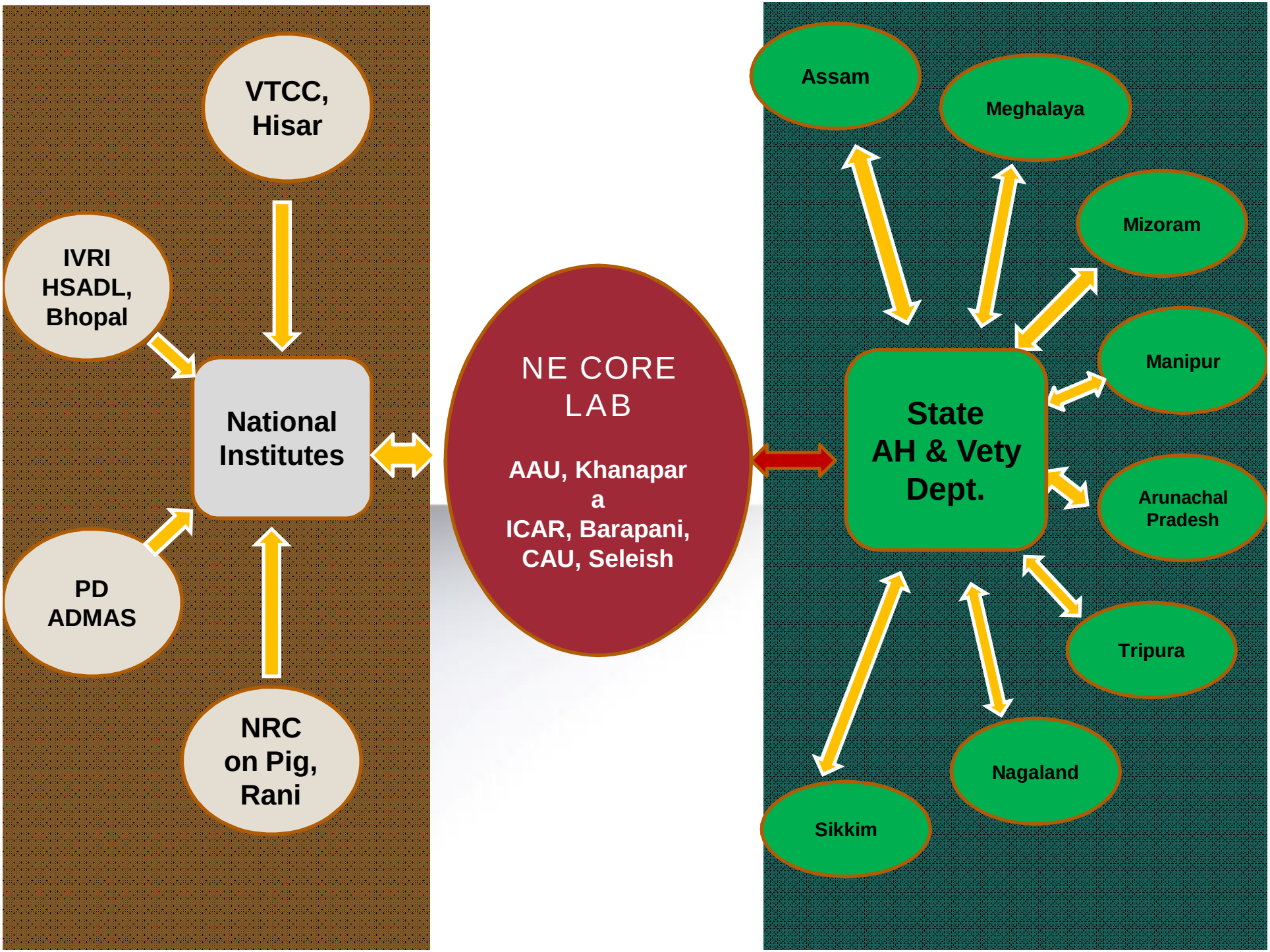
Dr. S. K. Das
Professor & Head
Dept of Microbiology
C.V.Sc, Khanapara

Partners

- **Northeast Institutes**
  - College of Veterinary College, Assam Agricultural University, Khanapara
  - Animal Health Division, ICAR Research Complex for NEH Region, Umiam, Meghalaya
  - Central Agricultural University, Selesih, Aizawl

- **National Institutes**
  - Veterinary type Culture Collection (VTCC), NRC on Equines, Hissar
  - High Security Animal Disease Laboratory, IVRI, Bhopal
  - Project Directorate on Animal Disease Monitoring and Surveillance, Bengaluru
  - National Research Centre on Pig as collaborating centre

- **State agencies**
  - State Animal Husbandry Department of states of Northeastern states
  - Assam, Meghalaya, Tripura, Manipur, Nagaland, Mizoram, Arunachal and Sikkim……



Objectives

- **Research on transboundary and endemic animal diseases of NER, so as to develop core competence on rapid diagnosis and control of such diseases.**

- **Training of the State Veterinary personnel on the importance and periodicity of disease reporting, use of advanced / molecular diagnostic kits to detect diseases and sampling techniques.**

Objectives……

- **Development of human resources in the form of PG level students to man this sector technologically using frontier technologies. Capacity building of the core faculty in advanced laboratories of the world.**

- **Development of a public – public partnership module encompassing three public partners for effectively handling the animal – man – environment continuum chain.**

Mode of work

STUDY

ANALYZE

PREDICT

IDENTIFY

RESPOND

PREVENT

PREPARE

DELIVER

Role of partners at Regional level

- Development of diagnostic protocols, kits and competence.
- Identification of disease prevalence zones along with migration patterns through GIS, regular sero-monitoring and molecular epidemiological studies.
- National and international linkages on information, technology and human resource sharing leading to value addition to the programme.

Role of partners at Regional level

- To collect, characterize and preserve sera, microbial isolates systematically at Regional (AAU), as well as national referral facility (VTCC).
- Deployment of trained paravets, preferably outsourced in consultation with state animal husbandry departments for genuine and regular sample acquisitions.
- Creation of online database for emerging, TADs and endemic diseases (and, if possible, exotic diseases for which country is at risk from NER borders)

Role of partners at National Level

VTCC/NRCE

- Sample reposition and database creation
- Confirmation of the identified isolates and their conservation
- Strengthening the NE core labs with advanced diagnostic protocols developed by VTCC/NRCE

Role of partners at National Level

IVRI-HSADL

- Validation and development of diagnostic assays for diseases prevalent in NER

- Collaboration in handling/identifying emerging and reemerging diseases
  - Training programs for scientists and technicians
  - Work pertaining to exotic diseases and diseases to be handled under conditions of maximum containment.

Role of partners at National Level

PDADMAS

- Active and passive surveillance of animal diseases in collaboration with NER institutes using molecular means
- Development of medium range animal disease forecasting system together with an animal disease decision support system for the NE region
- Epidemiological and surveillance related training to veterinary officers of the NE region

Role of partners at National Level

NRC Pig will associate in

- Collaboration with the core labs with respect to quick identification and development of management protocol for diseases relating to pigs
- Development of advanced diagnostics in the specific areas where expertise is available

Role of partners at State AH & Vety Department

- Sponsoring field veterinarians for capacity building in areas of molecular disease diagnostic techniques, appropriate sampling protocols and disease reporting.
- Creation of a suitable sampling strategy and regular sample collections for dispatch to core labs of NER.
- Timely reporting of diseases to the core laboratories

- GIS based disease tracking and utilizing core lab technical support in creating disease free zones/ containment zones particularly for transboundary diseases.

- Active collaboration in developing state specific disease control measures.

WORK PLAN - NER

- Surveillance of enzootic, emerging and TADs of livestock in the border states of the north east utilising validated and commercially available technologies.

- Development of diagnostics for rapid detection – preferably pre-clinical diagnosis within a reasonable timeframe

Identification of disease prevalence zones through regular sero-monitoring, GIS and molecular epidemiological study

Endemic infectious agents

Bacterial agents - *Brucella, Pasteurella, Leptospira, Salmonella*, *Mycobacterial* species.

Viral agents – CSF, Porcine parvo virus, porcine circo virus, rotavirus, IBR, PPR, Blue tongue virus, IBDV, Duck plague virus.

Parasitic agents - *Fasciola, Paramphistomum, Schistosoma, Moniezia* and larval cestodes, Gastrointestinal nematode, *Coccidia, Babesia, Theileria, Anaplasma, Trypanosoma*.

Exotic/ TADs agents

- **Bacterial agents - Methicillin resistant staphylococci, Carbapenem-resistant E.coli, Oedema disease, multi- drug resistant tuberculosis.**
- **Viral agents – Nipah virus, PRRS virus, Swine influenza, PED virus & TGEV, Buffaloe, JEV, WeNV & swine pox**

WORK PLAN – National Institutes

- Development of protocols and techniques for detecting economically important diseases of livestock in collaboration with HSADL-IVRI, PD_ADMAS and VTCC-NRCE
- Development of a disease database on enzootic, emerging and re-emerging diseases (surveillance-based) as well as for TADs (Pest Risk Analysis-based) in collaboration with PD-ADMAS
- Development of indigenous diagnostics at centers of North east and National Institutes

WORK PLAN – National Institutes

- Development of an effective and workable mechanism for disease reporting in the region, which may serve as a model for similar agroecological regions (transboundary-areas) located across the country.

- Strengthening of the regional repository at AAU, Khanapara through the expertise of VTCC.

WORK PLAN – National Institutes

- Setting up of a core NE cell and a team to deal with the implementation of the project
- Formulation of a dedicated schedule of activities pertaining to the project
- Devising a timeframe for implementation of the specific objectives and regular liaison with the NE counterparts
- Developing a diagnostic facilitation and a validation center as part of this programme
- Developing training modules for NER pertaining to this programme
- Periodic visits and interaction of experts to the NER.

Work plan- State Animal Husbandry/Vet Department

- Creation of a nodal team for effective interfacing and ensuring implementation of project.

- Preparation of disease sampling zones/grids in consultation with respective core labs. Creation of cold chain holds at critical places in the state for sample storage.

- Facilitating training of veterinarians and disease investigation officers at the designated core labs.

Ensuring support to the paravets and related veterinary personnel for sample collection.

Regular and timely dispatch of samples to the core labs .

Incorporating disease related information in the state disease database and monthly outbreak reports.

Enforcing disease restriction/quarantine measures if deemed fit from time to time.

Basic working plan and linkages

Network & Work Flow Diagram

VTCC, HSADL and PD ADMAS,

eIADs
Disease informati on

Nodal agencies

NE Core Labs

ICAR

DAHD&F, MOA, GOI

OIE

SAHD, NE States

SAHD, NE states

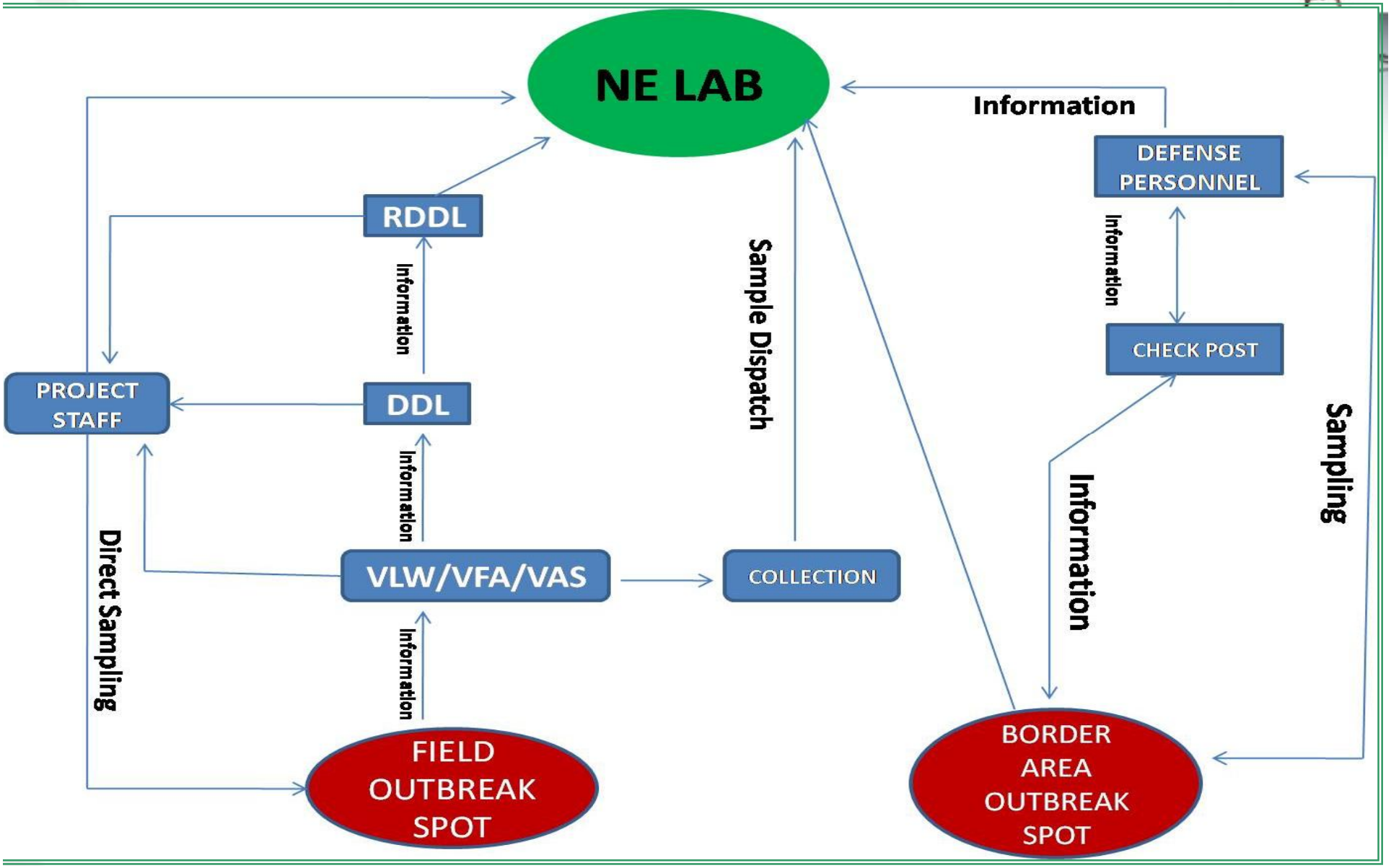
Disease investigation officer

Paravets

Block Veterinary Officer

Village clusters, Nomadic rearers, Farms, Backyard holds

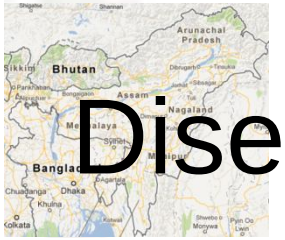
WORK PLAN - Sampling



WORK PLAN – Education & Training

Customized, need based training modules

Modules	Trainers	Trainees
• Sampling • Data entry • Transportation • Preliminary and advanced diagnosis • TAD importance • Epidemiology • Surveillance • Data analysis • Software management • Reposition • OIE protocols • Basic microbiology	• NE labs • NE Labs/PDADMAS • NE Labs/VTCC • NE Labs/ IVRI-HSADL • IVRI-HSADL/VTCC • North East labs/ State AH Deptt.	• State Animal Husbandry personnel • Contractual staff of core labs • North east scientists (Advanced overseas training) • State Animal Husbandry Disease investigation officers • Paravets



Diseases to be addressed….

	Porcine	Bovine	Ovine/Caprine	Poultry
Viral	CSF, PRRS, PPV, PCV, JEV, Rota, Nipah	BVD, IBR	PPR, Blue tongue, Goat/Sheep pox,	NDV, IBD, AIV, Mareks disease, Adenovirus
Bacterial	Brucella, Salmonella, E.coli, MDR bacteria	Brucella, Tuberculosis, Mastitis, MDR bacteria	Brucella, E.coli, Pasturella	Salmonella, E.coli
Parasitic	*Fasciola, Paramphistomum, Schistosoma, Moniezia* and larval cestodes, Gastrointestinal nematode, *Coccidia, Babesia, Theileria, Anaplasma, Trypanosoma*			
Core Mandates	Diagnosis Training Reposition Disease mapping	Diagnosis Training Reposition Disease mapping	Diagnosis Training Reposition Disease mapping	Diagnosis Training Reposition Disease mapping

Activity break up and issues regarding implementation

	Issues	Programmes	Activity	Way Forward
Diagnosis	Infrastructure Competence Frequency	AsCAD, State D.I Programs, PD ADMAS, DBT networks	Regional level disease diagnosis to be carried out in a phased manner	Decide on sample despatch schedule. Decide on the phased programmes of disease surveillance.
Training	Schedule Need based Manpower fidelity	TSP, State DI programs, DAHDF programs	Regular trainings to vets and paravets	Create a training calendar
Reposition	Regularity Mode of transport Credit sharing			
Disease mapping	Identifying areas to be covered Zonation Disease coverage in phases	PDADMAS programme on disease surveillance	Creating a state disease geo spatial atlas	Enable GPS based zonation and sampling frames

Time Frames…..

	State DI Labs (A)	NER Core Labs(B)	National labs (C)	Linkage
Sampling	Twice a month	Once a month	Once a month	A-B
Report compilation	Once a month	Once a month	Once in six months	A-B-C
Diagnostic enabling	Every two months	Every Two months	To be reviewed once a year	A-B-C
Training	Once in six months	Once in six months	Once in six months	A-B-C
Disease Mapping	Every Two months	Once in six months		A-B
Reposition		Every month	Once in six months	B-C
Data analysis		Once in six months	Once in six months	B-C

Core Lab jurisdictions…….

- **Assam Agricultural University, Khanapara-** would mentor the states of Assam, Sikkim and Arunachal Pradesh.

- **ICAR Research Complex for NEH, Barapani-** would mentor the states of Meghalaya, Nagaland and Tripura.

- **College of Veterinary Sciences, CAU, Selesih, Aizawl-** would mentor the states of Manipur and Mizoram.

Expected Outcome……..

- Creation of Core facilities across the region in terms of State-of-the-art modern laboratories ready to handle exigencies.
- Creation of disease incidence and zone maps- Early Warning System for TADs
- A critical mass of human resource (scientists, veterinarians, technicians etc) trained in eTADs ready to be deployed in need
- Technologies/Products in the form of a battery of diagnostic assays/kits, vaccines, bank of serum samples and clinical samples

Expected Outcome……..

- New knowledge and information on disease, pathogens, epidemiology and a scientifically validated Disease Database
- Addition of the isolates to VTCC / AAU repository as a national resource
- Establishment of research linkage with national and international institutes
- Establishment of surveillance and sampling network

EXPECTED OUTPUTS ……..

• Creation of nodal units to cater the prospective and retrospective disease incidence/identification in terms of reliable diagnostic competence in the NER.

• Development of animal health management package in the NER through early warning systems and reliable diagnostic assistance for effective control of diseases

• Address problems of disease diagnosis through the deployment/development of user friendly disease diagnostic kits in the NER.

Research agenda for the NE core labs…

Microbial diseases:

- **Development of molecular diagnostic tools for detection of CSFV.**
- **Development of user friendly tests using genomic and proteomic tools for detection of Duck Plague Virus.**
- **Development of Improved Diagnostics for rapid detection and characterization of rotaviruses associated with piglet diarrhea.**
- **Development of a rapid multiplex PCR assay for serotyping of common clinical *Salmonella* isolates.**
- **Molecular epidemiology of economically important animal viruses like arboviruses, PCV, PPV, CSFV, IBD, NDV and PRRS.**
- **Development of antigen cartography models for surveying antibody variant status against CSFV, PPV and PCV.**
- **Development of models for correlating antibody status and pathogen load in cases of persistent /chronic infections by Real time PCR.**
- **Studies on cytokine cascades and role of gamma interferons in CSFV and NDV.**

Research agenda for the NE core labs…

Parasitology/ Hemoprotozoal Diseases

- Development of molecular diagnostic tools for detection of porcine cysticercosis.
- Development of molecular diagnostic tools for detection of bovine babesiosis, trypanosomiasis and theileriasis.
- Prevalence study and epidemiology of gastro-intestinal parasites of animals and birds of NER.

Public Health

- **I**dentification, surveillance and documentation of drug-resistant *Mycobacterium bovis* in North-Eastern Region of India.
- **H**orizontal and clonal spread of resistant bacterial genes among animal – plant – human species.

Research on New generation Vaccine Development

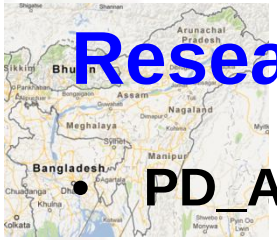
Research Agenda for National institute……

VTCC/NRCE

- Influenza A virus
- B) Japanese Encephalitis/west Nile virus
- C) Trypanosomiasis

IVRI-HSADL

- A) Development of Multiplex DNA-Strip test for ALL prioritized BSL-III/IV pathogens
- B) Development of type-specific antigen capture ELISA for detection of AIV in poultry with
- reference to the NER
- C) Development of quantitative multiplex real-time RT-PCR for the simultaneous
- typing and sub typing of Influenza type A viruses in the NER
- D) Development of Real-Time PCR for detection of Malignant Catarrhal Fever infection in domestic animals in NE India
- E) Development of diagnostics for detection of West Nile virus infection in wild resident and migratory birds in India.
- F) Development of an isothermal amplification method for rapid detection and typing of Porcine Reproductive and Respiratory Syndrome Virus infection in pigs



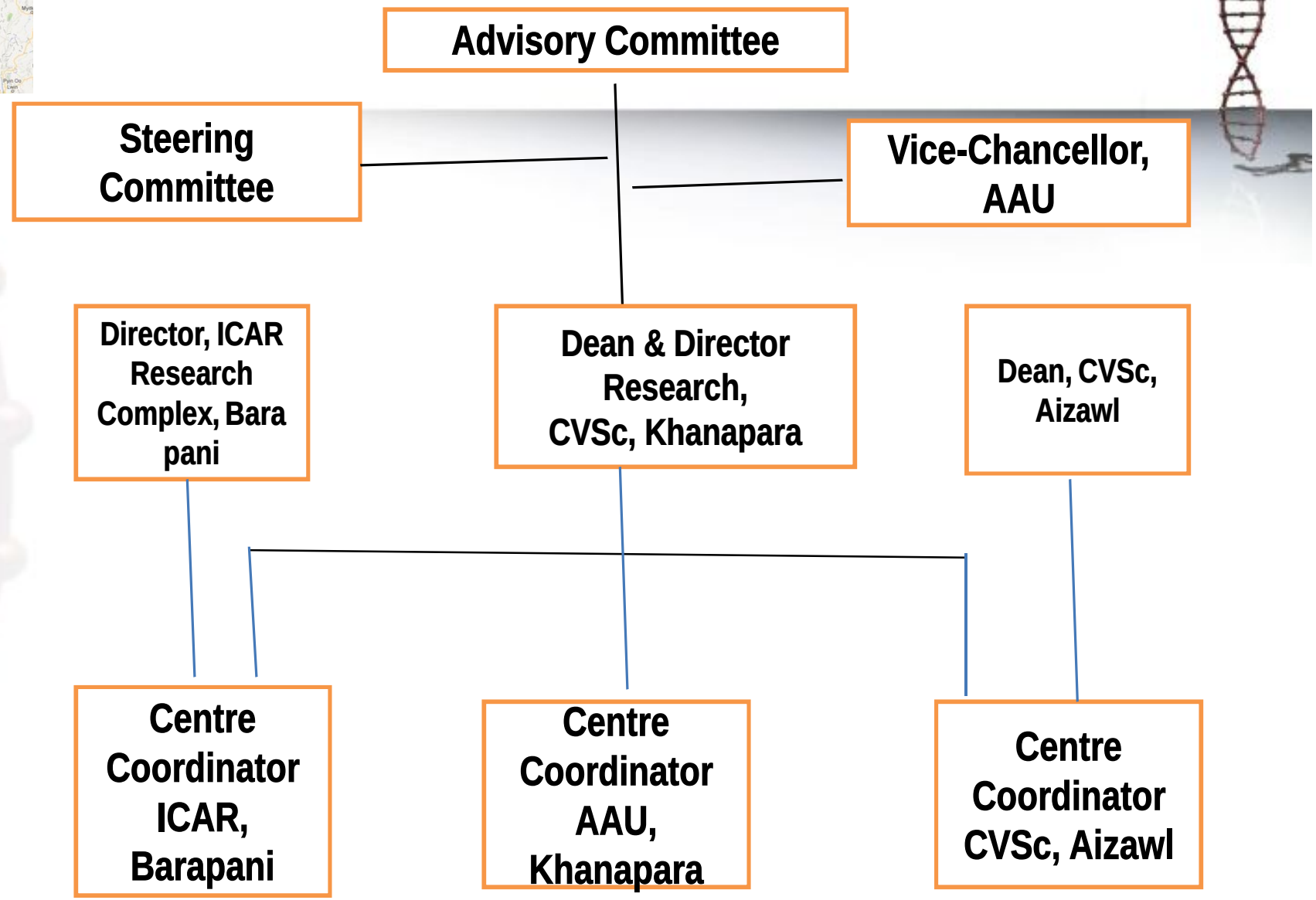
Research Agenda for National institute……

- **PD_ADMAS**
- Development of Infectious Disease Information System (IDIS) and Risk assessment models for Transboundary animal diseases (TAD) & other emerging diseases in NE region of India
- **<u>Development of New Generation diagnostic tests</u>-**
- Pen side tests (Immunochromatographic lateral flow tests), Multiplex PCR (real time), Immuno-comb test, Dip stick ELISA and Latex agglutination test, Loop mediated isothermal amplification (LAMP), IDT AST estimation by Phoenix-100/Vitek/automated IDT AST testing system, Rapid immunoprecipitation assay, Maldi- Biometry.
- **<u>Immunoserological-</u>**
- Pen side tests (Immunochromatographic lateral flow tests), Immuno-comb test, Dip stick ELISA and Latex agglutination test, and Rapid immunoprecipitation assay.
- **<u>Nucleic acid based-</u>**
- Multiplex PCR (real time), Loop mediated isothermal amplification (LAMP), IDT AST estimation by Phoenix-100 coupled with RT PCR.

Activity Milestone:

Sl No	Project Activity	Year1	Year2	Year3	Year4	Year5
1	Building Design					
2	Building Construction					
3	Procurement of Equipment					
4	Recruitment of Permanent Staff					
5	Recruitment of Project Staff					
6	Research Activity					
7	Diagnostic Production at AAU, Khanapara , validation					
8	Training of lab personals, Diagnostic Products available at DIO labs					

Proposed Organizational Structure



Composition of the Advisory Committee (AC):

- Vice-Chancellor, AAU, Jorhat – Chairman
- An Eminent National/International Scientist of relevant expertise to be nominated by Chairman in consultation with Steering Committee – Co-Chairman
- Adviser, DBT & Nodal Officer, NER-BPMC – Member
- Three Scientists of National Repute to be nominated by Chairman in consultation with Steering Committee – Members
- Head of the Institutes [In AAU Dean & DR(vety) both]
- Co-ordinator, Lead Centre, Advanced Animal Disease Diagnostics and Service Management Centre – Member Secretary
- Centre Coordinators - Members

Composition of the Steering Committee (SC)

- Dean, AAU, Khanapara – Chairman
- A DBT Nominee – Member
- Directors of IVRI, HSADL, VTCC & PD-ADMAS - Members
- Directors of AH & Vety. Depts. of NE States – Members
- Heads of the Institutions of respective institutes (In AAU DR Vety) – Members
- Co-ordinator, Lead Centre, Advanced Animal Disease Diagnostics and Service Management Centre – Member Secretary
- Centre Coordinators – Members
- Coordinator of Collaborating Centre - Member

Centre Management Committees (CMC)

- DR (Vety), AAU – Chairman
- HoDs of related depts. (Microbiology, Biotechnology, Pathology, Parasitology and Public Health) – Members
- One DBT nominee – Member
- One nominee of the Directors of AH & Vety. Depts. of the concerned states of NE - Member
- Centre Coordinator – Member Secretary

Monitoring mechanism

By way of three committees viz,

1. **Advisory Committee**
2. **Steering Committee**
3. **Centre Management Committees (CMC)**

BUDGET

Head of expenditure	AAU, Khanapara (A)	ICAR, Barapani (B)	CoVSc, Selesih (C)
Non Recurring	1143.17 (Equipment+Power back up+ Computer+ Lab +BSL3+ Animal House)	68.99	88.44
Recurring, Manpower	167.33	67.92	53.40
Recurring Consumables and Maintenance	219.00 (Consusum+Train+Student+Travel+Cont+AMC+Over Head)	96.08	96.00
Total Cost	**1529.50**	232.99	237.84
Total Cost of Proposal of NER Labs (A+B+C)	**2000.33 Lakhs**		

BUDGET IN LAKHS (Rs.) FOR AH & Vety Deprtments

A. Non-Recurring
(Equipments+ Strengthening of Labs) : 238.80+ 221.63+155.63= Rs. 616.06 Lakhs

B. Training of Veterinary officers, field assistant, Lab technicians : Necessary TA/DA will be arranged by respective Core Lab of NER

C. Diagnostic kit, reagents, plastic and glass wares will be arranged by respective Core Lab of NER

D. TA : @1 lakh/state/yr 12.00 + 12.00 + 8.00 = Rs 32.00 Lakhs

E. Contingency : @1 lakh/state/yr 12.00 + 12.00 + 8.00 = Rs. 32.00 Lakhs

Over Head : @ 0.50 lakh/state/yr 6.00 + 6.00 + 4.00 = Rs. 16.00 Lakhs

D. Total :Rs 268.80(Ass) +Rs 251.63(Bara) + Rs 175.63 (Izaw) = Rs. 696.06 Lakhs

DISTRIBUTION OF FUNDS

Sl No	Name of the centre	Amount (In Crore)
1	AAU, Khanapara Core Lab	Rs. 15.295
2	ICAR Barapani Core Lab	Rs. 2.3299
3	CAU, Aizwal	Rs. 2.3784
4	PD-ADMAS, Bangaluru	Rs. 2.8905
5	VTCC/NRCE, Hisar	Rs. 3.6716
6	HSADL, IVRI, Bhopal	Rs. 2.3149
7	NRC on Pig	Rs. 0.9297
8	AH & Vety. Departments of 8 states	Rs. 6.9606
Grand Total		Rs 36.7706

Thank you

Animal Disease Monitoring and Surveillance

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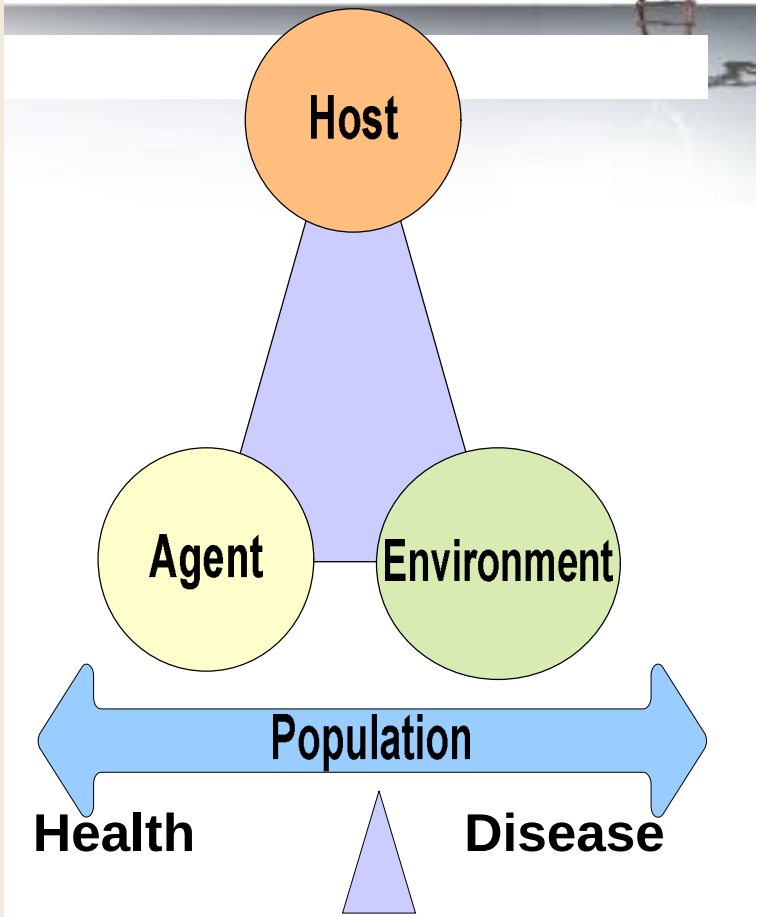
Disease Results From Interaction

- ***Agent*** **(etiology) and**
- ***Susceptible host*** (livestock population)
- ***Environment*** - supports transmission of agent

E**xtrinsic factors** that affect agent and the host and opportunity for exposure are

- **Geology and climate,**
- **Biological factors** - insect vectors
- **Socioeconomic and Livestock rearing practices** crowding, sanitation, and health services



Disease monitoring and surveillance

- **Surveillance and monitoring are often used interchangeably but not alike**

- **OIE defines :**

Surveillance **- continuous investigation of a given population to detect the occurrence of disease for control purposes, which may involve testing of a part of the population.**

Monitoring - **constitutes on-going programs directed at the detection of changes in the prevalence of disease in a given population and in its environment.**

Surveillance and monitoring

Surveillance

- Transforms data into information
- Implies an action
- Essential for diseases under a program

Monitoring

- Overview of disease occurrence
- Does not imply an action
- Basis for the development of a program

Both activities require the support of competent diagnostic laboratories

Surveillance encompases-

- **Infectious agent, host, reservoirs, vectors, environment, disease spread pattern.**
- **Evaluation of morbidity and mortality data, control measures, assessment of immunity, etc.**
- **Laboratory techniques that revolutionized and are integral component of surveillance.**
- **However, surveillance process is costly when several diseases considered at one time.**

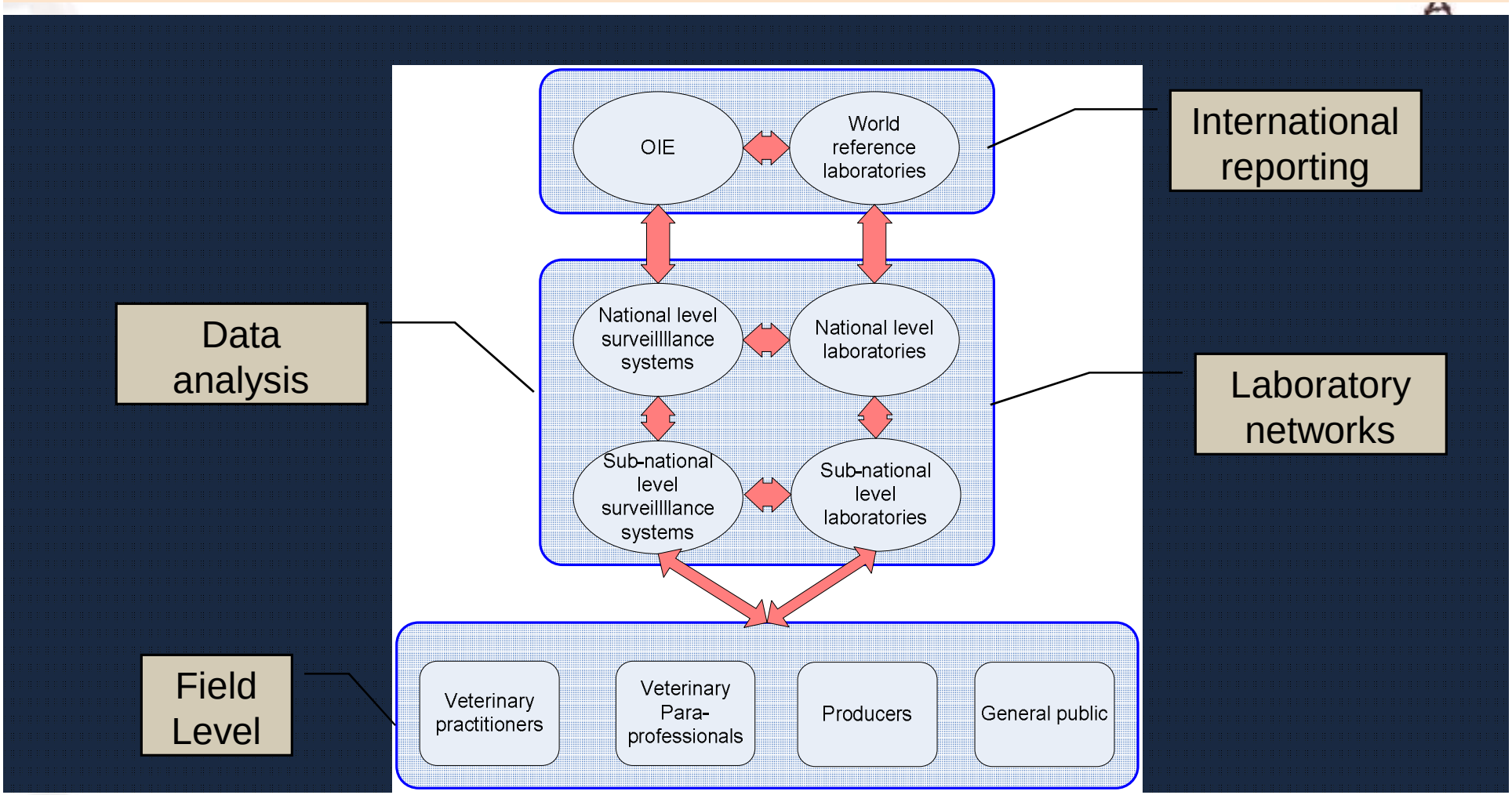
Why surveillance?

- To collect, analyze and interprete data so as to determine distribution of diseases including exotic/new in time and space and to confirm presence or absence of disease.
- To establish a hierarchy of importance (economic or health or both) of a disease by recording incidence, prevalence, economic losses, etc. and directed at for control and eradication of disease.
- For evaluation of control programs.
- Monitoring of risk factors and other information.

Who Needs surveillance information and why

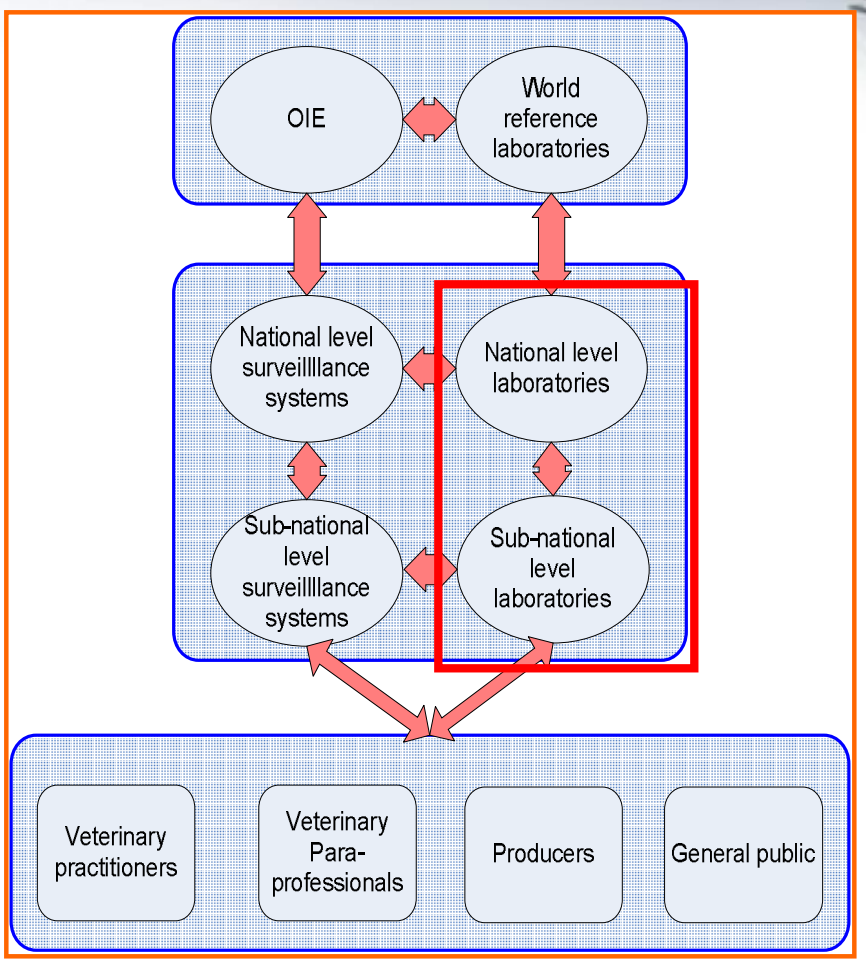
- Policy makers
  - to develop policies, disease control programs, and regulations
- Technical decision makers/Planners
- Veterinary service providers
  - input supplies, service planning/communication

Surveillance systems

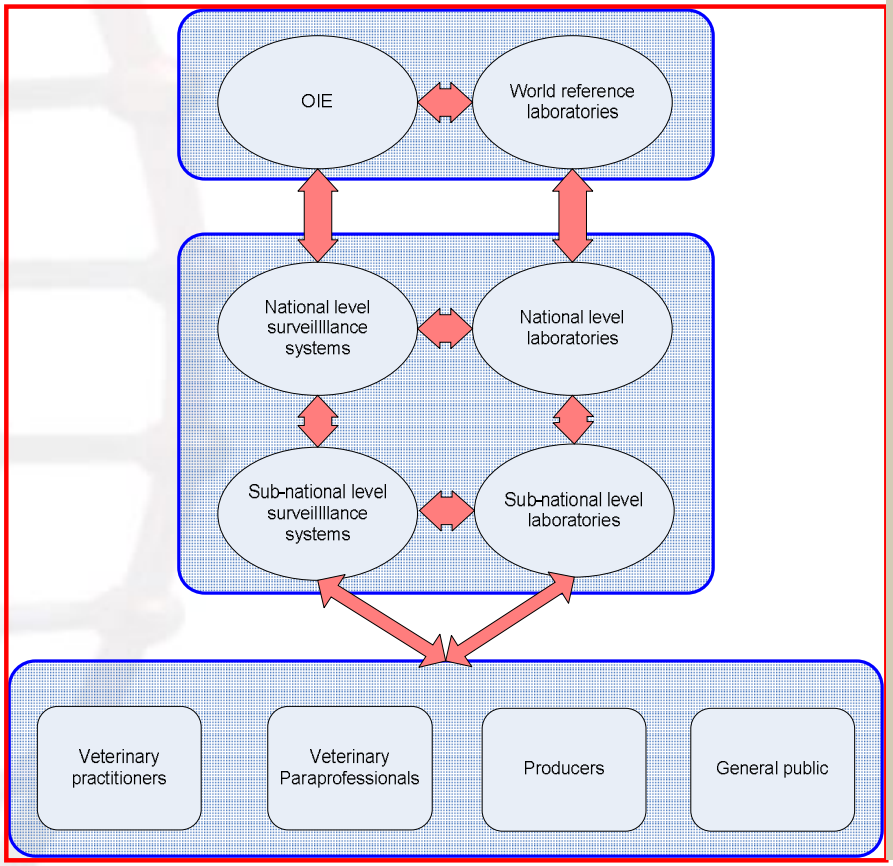


Diagnostic capabilities

- Fewer veterinarians have an interest for lab
- Specify the role of the veterinarian in the lab
- Increased dialogue between epidemiologists and the lab
  - Eliminate the “us and them” mentality



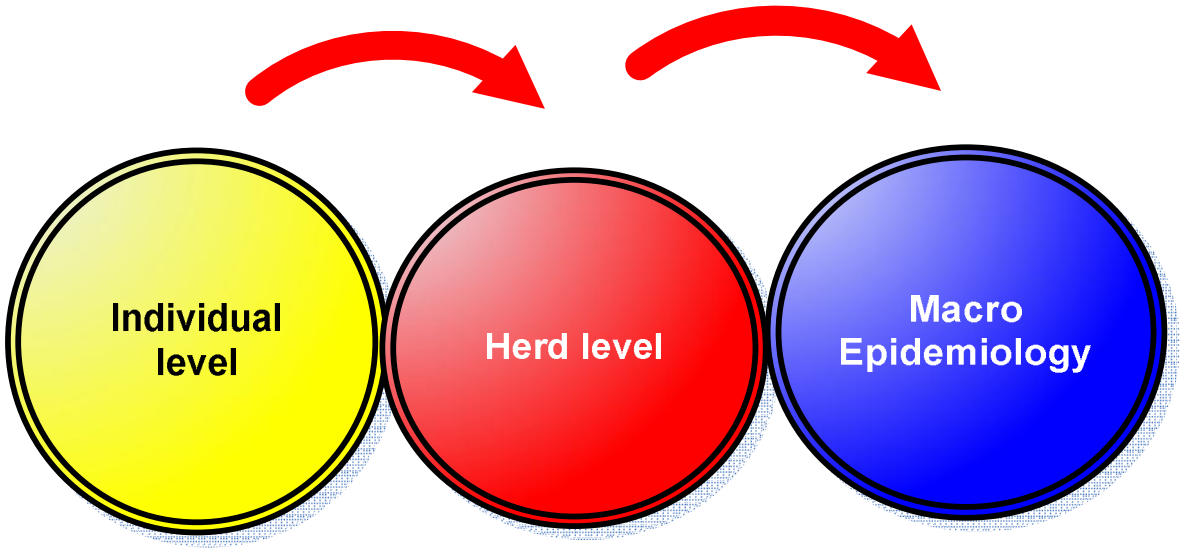
Increased understanding of



- Population based approaches
- Estimation of population parameters
- Interpretation of diagnostic tests
- Understanding of surveillance objectives and approaches
- Link to public health

Population based approaches

Need to shift from individual clinical case emphasis to broader population-based thinking



Passive surveillance

Can be used for

- Identification of diseases
- Disease location ;
- Respond to disease outbreaks;
- Meet the basic disease reporting requirements.

cannot be used for

- Determine the level and geographic patterns
- Setting priorities for disease control;
- Plan, implement and monitor disease control programs

Passive Data

- In India one Vet. Dispensary - one Vet covers a minimum of 10-15 villages.
- It is humanly impossible for the Vet to visit all the villages under his/her jurisdiction every day.
- She/he will visit only those villages from where he receives information of a disease.

So what She/he report is

PASSIVE DATA

Passive Data

Over reporting

Under reporting

- **Not confirmed by lab. analysis.**
- **Diagnosis is based on clinical signs only**
- **Differential diagnosis is not generally carried out**

Questionnaires used for collection of data

Name of the farm Owner……Phone No……Date……

Village / sub division/ District……Veterinarian / VHW ……

FARM ATTRIBUTES

1.1 Geographical attributes – *Where is the farm* ***located? Tick the box that more characterizes the location.***

☐ Dry Plan ☐ Flood Porn ☐ Hills ☐ Forest

1.2 Is the farm subjected to flooding during the wet season?

☐ Yes ☐ No

1.3 a.Production cycle

☐ All-in-all-out ☐ Continuous Production Cycle

1.3 b.Production cycle

☐ Close Herd (Farrow to Finisher)

☐ Finisher Site (Weaners to Finisher)

☐ Breeding Site (Sow to Piglets for Sale)

1.4a Current type of farming (*tick all that apply*)- *How do I classify the farm?*

☐ Indoor ☐ Partially Outdoor ☐ Scavanging ☐ Back yard

1.4b Pig raising system

☐ Free ranging ☐ Tethering ☐ Pen type

1.5. The floor of the pens made of:

☐ Cement/Concrete ☐ Mud floor ☐ Sand ☐ Wood planks

ANIMAL ATTRIBUTES

2.1 Type of the pigs:

☐ Domestic ☐ Feral ☐ Wild

2.2 Breed of the pigs:

☐ Local ☐ Exotic ☐ Cross-breed

2.3 Number of pigs – *at present*

Pig category	Number at present in the farm	Numbers of pen occupied
0—3 month		
3 Month—1 Year		
1 Year – 2 Years		
Above 2 Years		
Boar		
Total		

BIOSECURITY

3.1 Proper Fences in the farm boundary:

☐ Yes ☐ No

3.2 Do you use any of the following methods of disinfection*(tick the appropriate box and fill the cells when required*

	Method
	Disinfectant foot-bath
	Washing hands
	Clothes/boots for use on farm only
	Spray, vaporizer
	No disinfection

3.3 People on the farm undertake other activities linked to the pig production sector?

☐ Yes ☐ No

3.4 Which persons do you allow on your farm?

☐ Middleman
☐ Slaughterhouse owner/Killing point owner
☐ Friends/Family that keeps pigs or linked to the pig production sector
☐ Other pig farmers
☐ VHW/Veterinarians
☐ Other.....
☐ Nobody is allowed on the farm

3.5 Do you slaughter pigs in your own farm?

☐ Yes ☐ No

3.6 Can fattened pigs be bought from other farm?

☐ Yes ☐ No

3.7 Purchased stock

☐ Piglets ☐ Gilts ☐ Boar ☐ Sow ☐ Semen

3.8 Origin of Purchased stock

☐ Live market ☐ Farm of other Breeder ☐ NGO ☐ Own Farm

3.9 Are you purchased gilts mixed with sow?

☐ Always ☐ Sometimes ☐ Rarely ☐ Never

3.10 How are the sows inseminated?

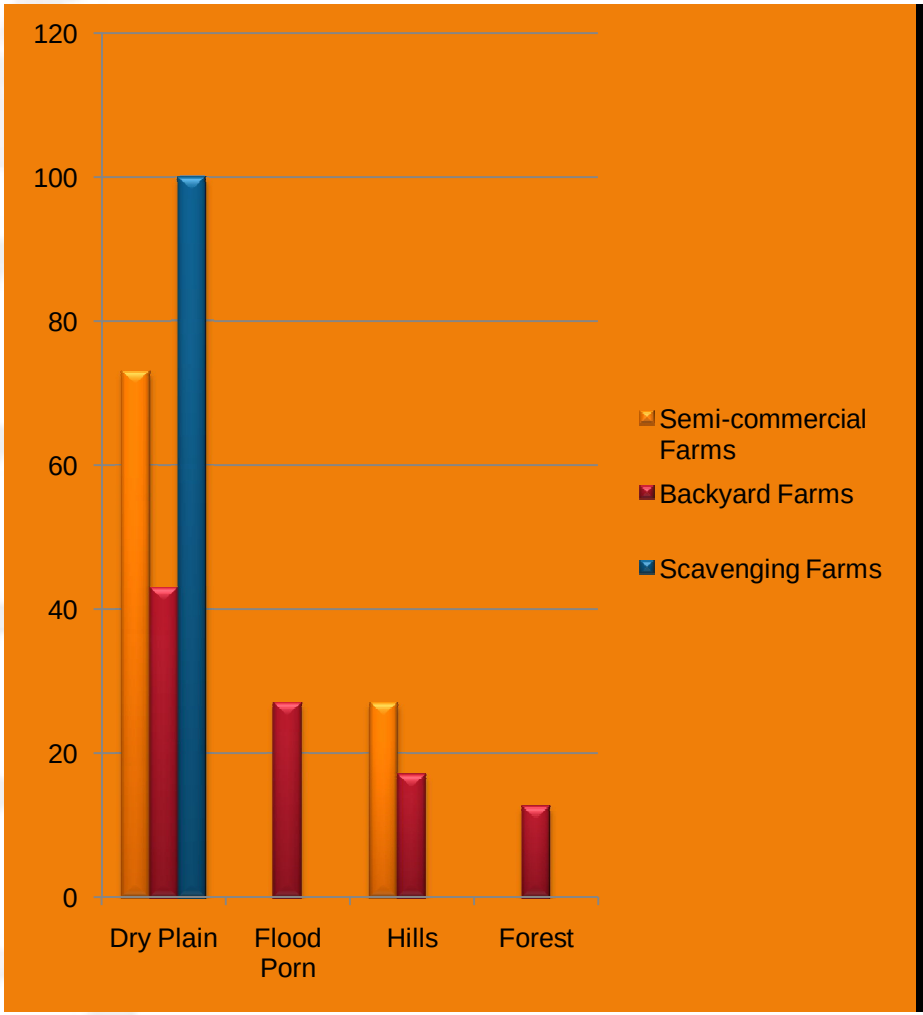
☐ Farm Boar ☐ Village Boar ☐ Wild Boar ☐ A.I.

4.0 If boars are present in the farm, are they also used for reproduction for different farms?

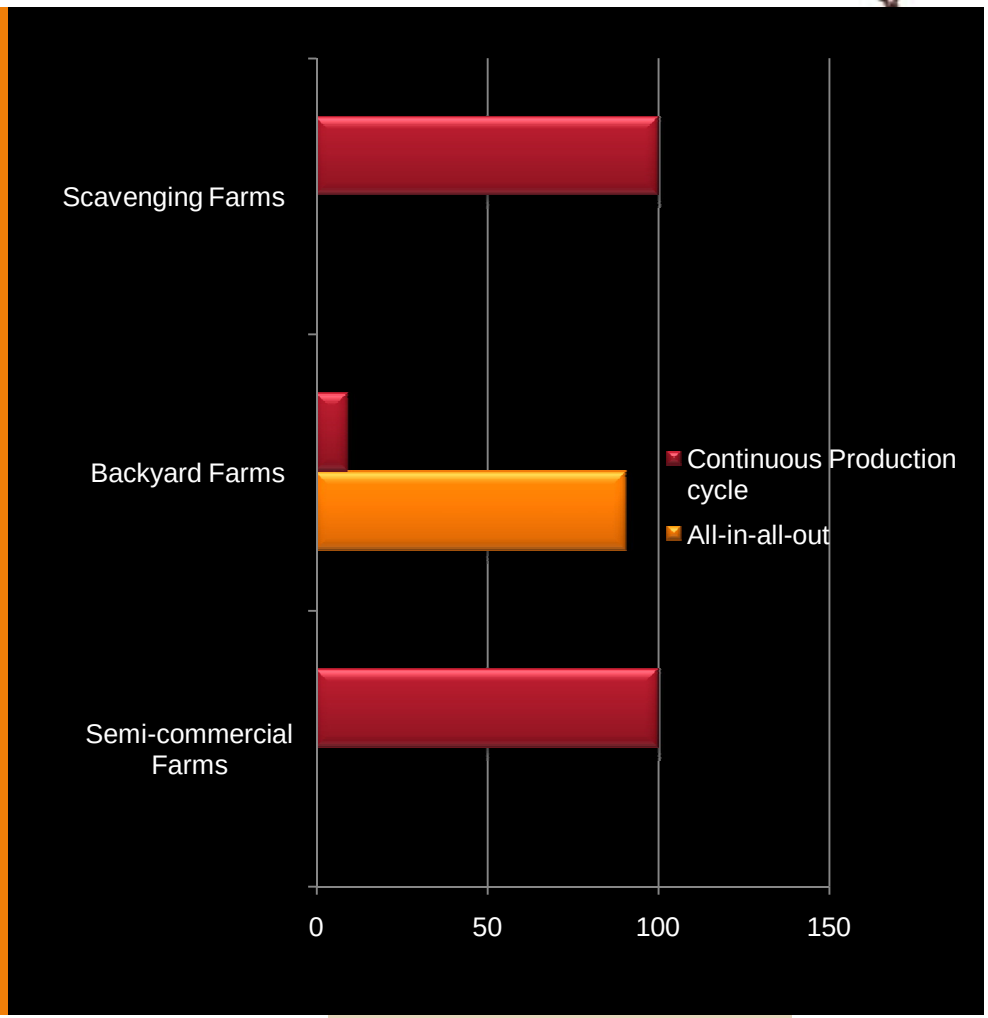
☐ Always ☐ Sometimes ☐ Rarely ☐ Never

4.1 Are other farm's boars brought in to give service to your sows?

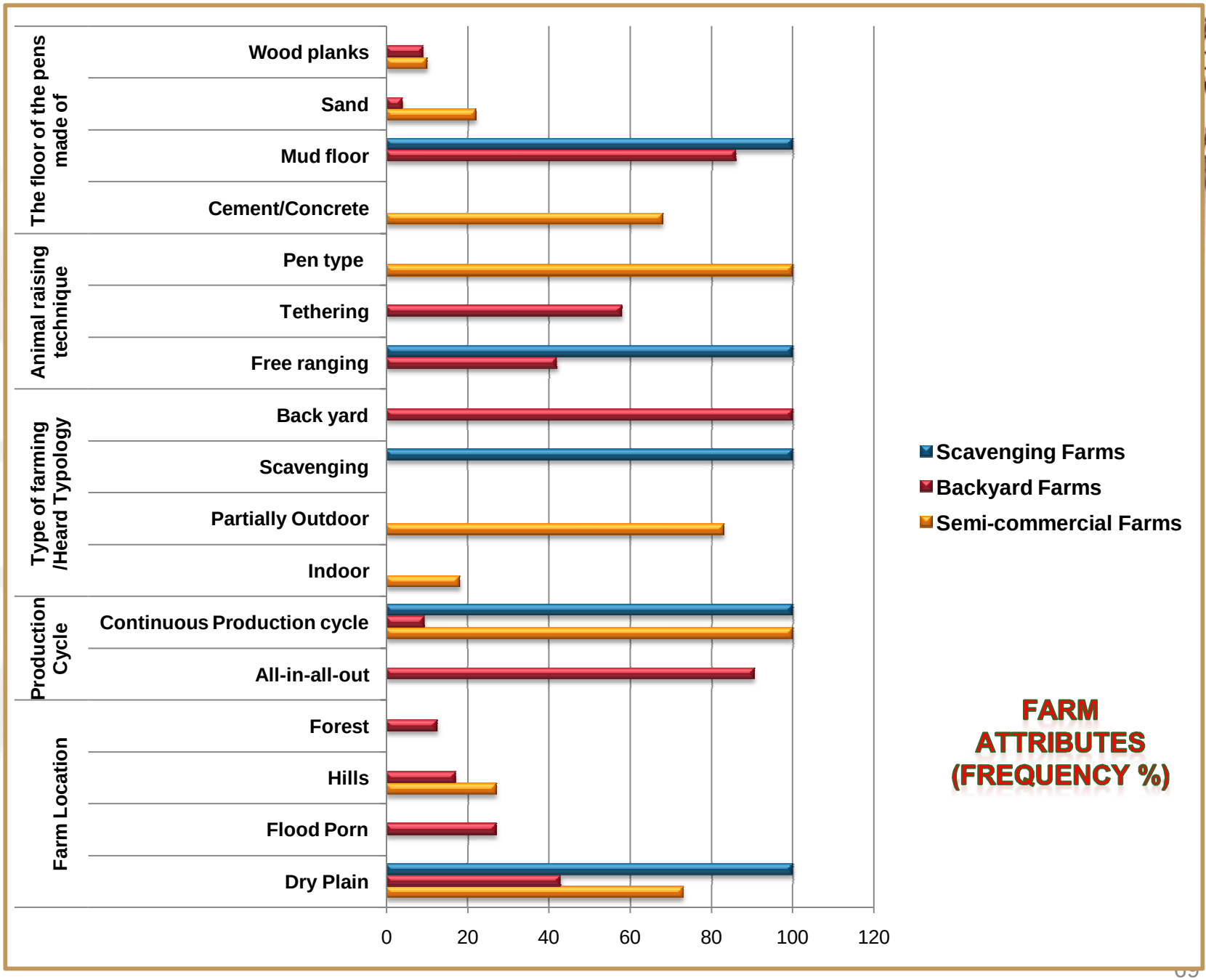
☐ Always ☐ Sometimes ☐ Rarely ☐ Never

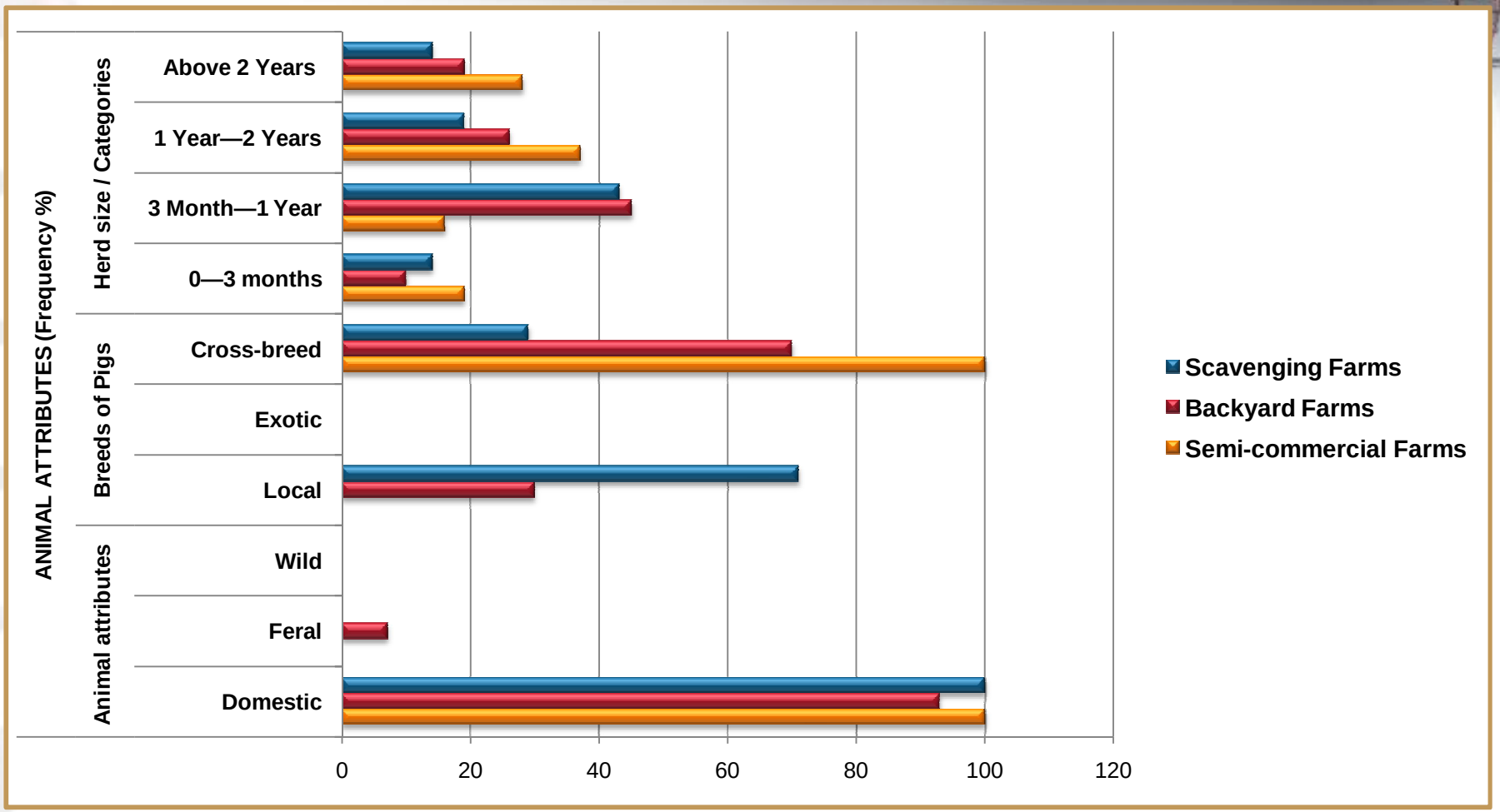


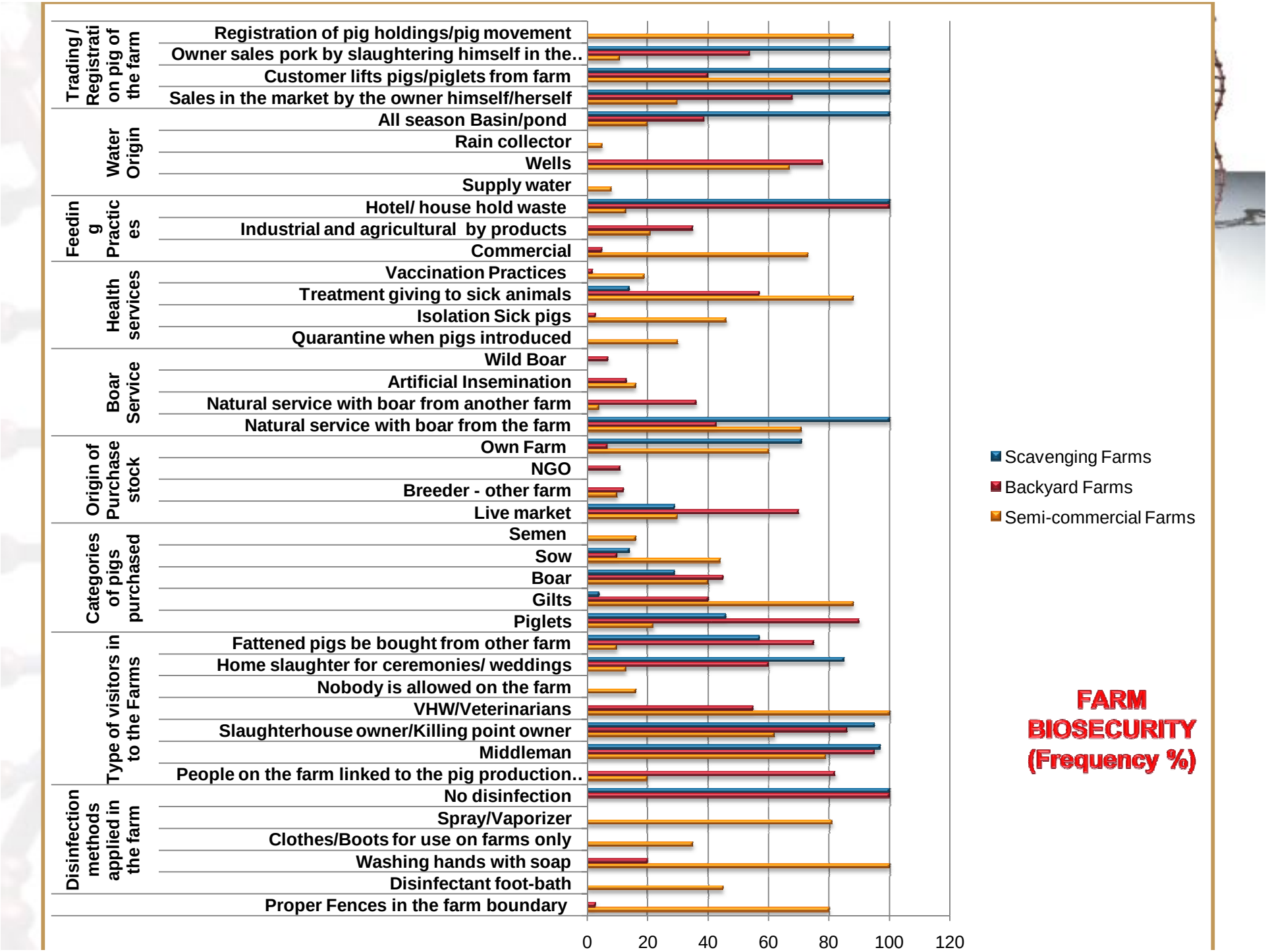
Farm Location



Production system







Knowledge, attitudes & practices (KAP Study)

- Many farmers didn't know vaccines were specific to particular diseases
- Not commonly used by village farmers
  - 20% had never vaccinated
  - Poor condition (cold chain)
  - Multiple use between villages
  - Often sold wrong vaccines
  - No accurate diagnosis

Knowledge, attitudes & practices (KAP Study)

- Attitudes to Vet Services – 30% didn't contact them
  - Only when severe disease or treatment ineffective (50%)
  - No use – ineffective (32%)
  - Too late; too long to come
  - Don't know or cannot contact them (>50%)
- Farmers were MUCH more likely to call a local animal health worker (unqualified) than a veterinarian

"Active Surveillance"

Veterinary Authority actively searches for evidence of disease

1. Survey of farmers using questionnaire
2. Survey of farmers using "participatory disease surveillance" tools (group or individual interviews)
3. Serological surveys
4. Abattoir observations
5. Investigates causes of reported cases or outbreaks
6. Samples collected and tested in diagnostic laboratory

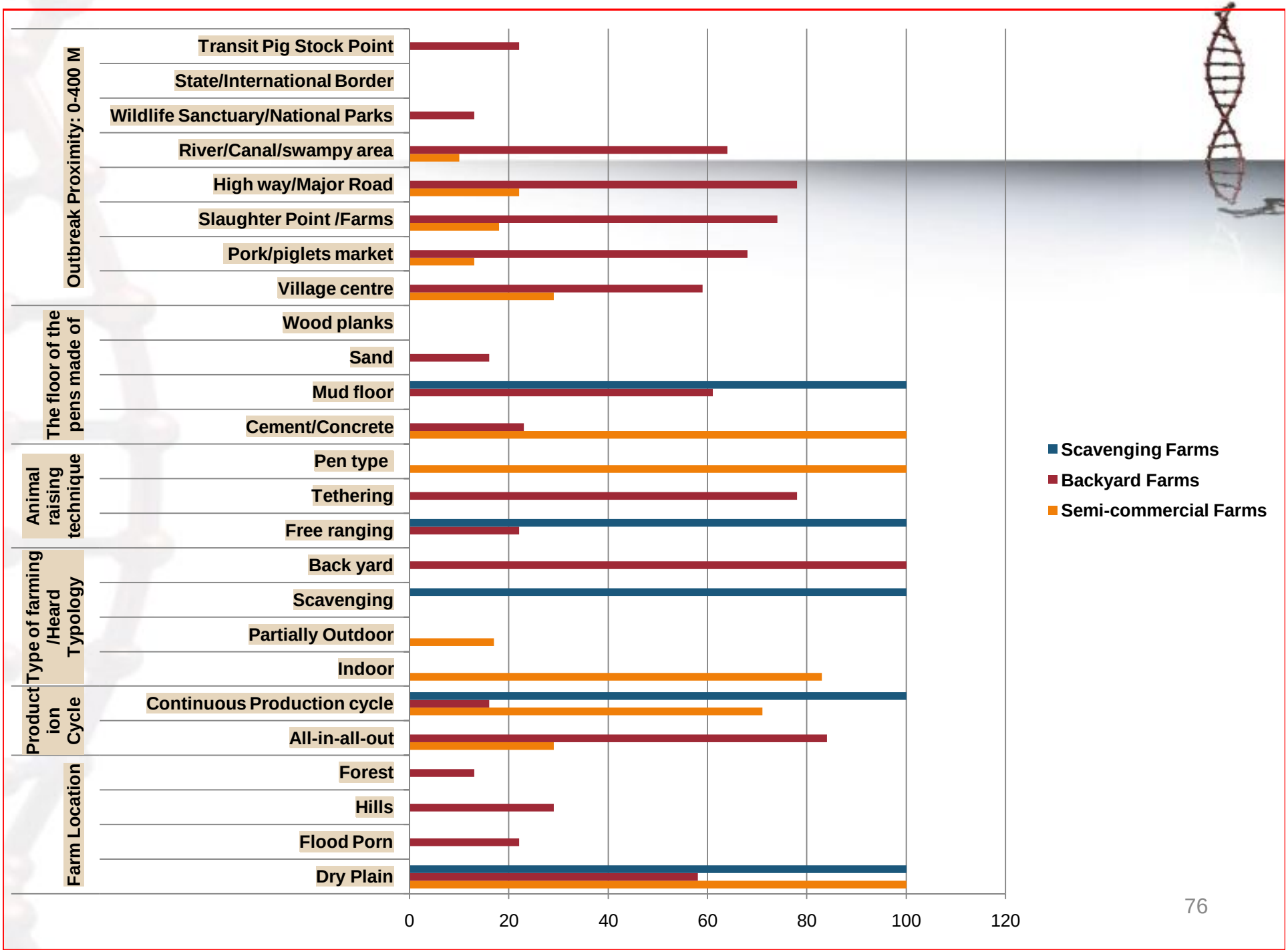
Active surveillance

Active surveillance must be able to address

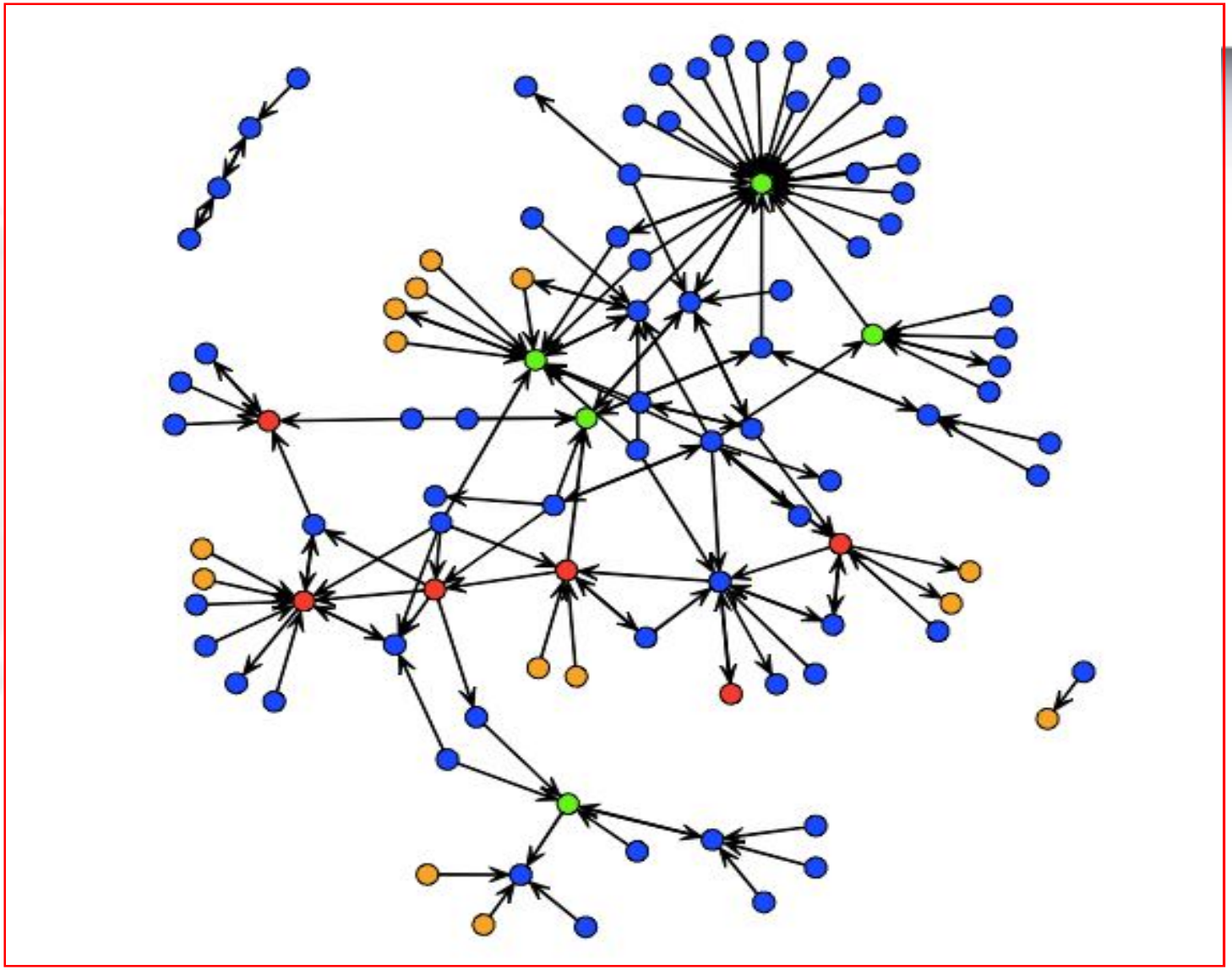
- **Problems of mis/over under reporting.**
- **True disease situation in a population.**
- **Population of a known size to allow the calculation of rates and proportions.**

Components of successful active surveillance

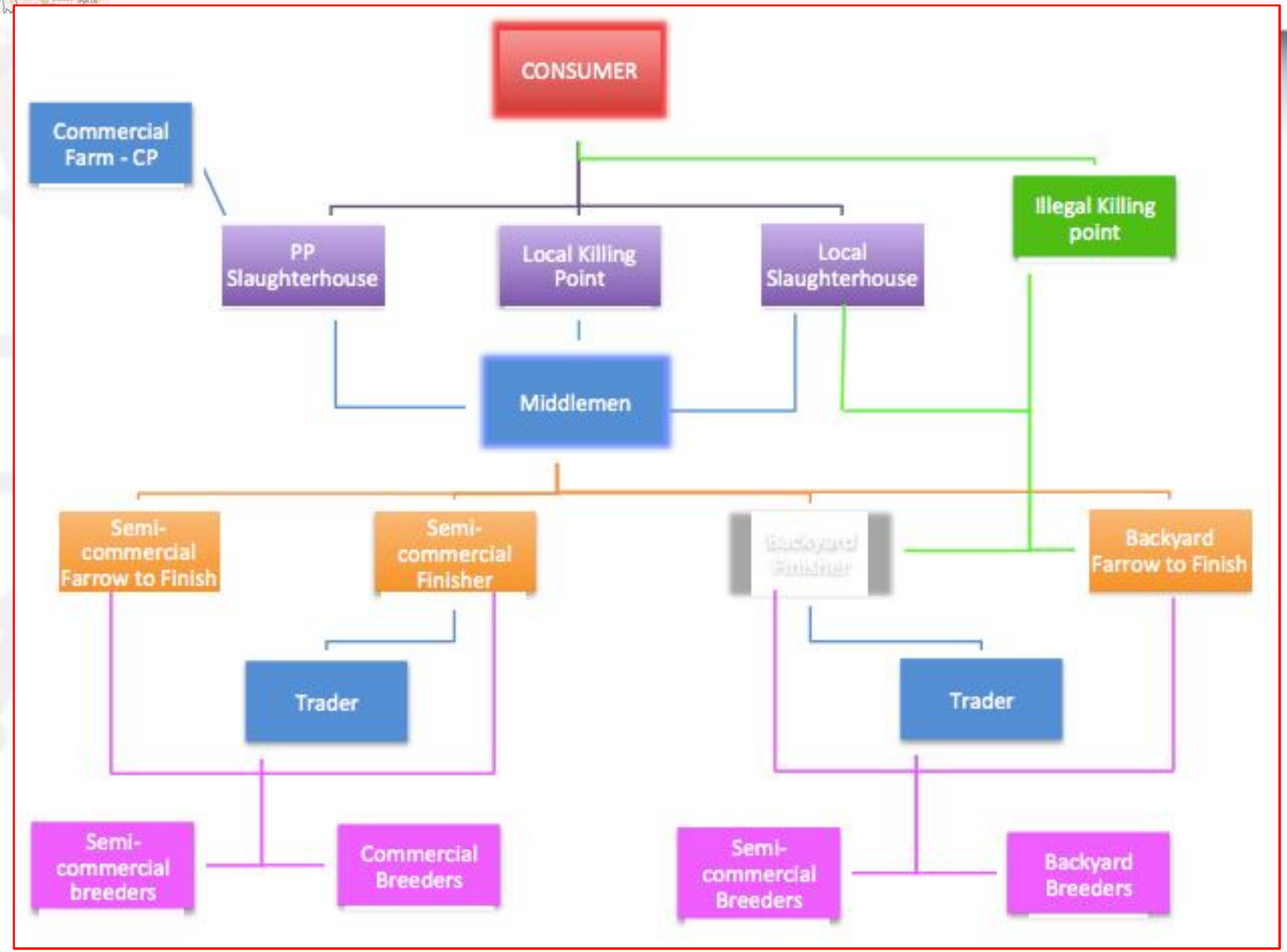
- **Identification of `high-risk' pockets;**
- **Integration - field and laboratory veterinary services;**
- **Regular visits to farming communities**



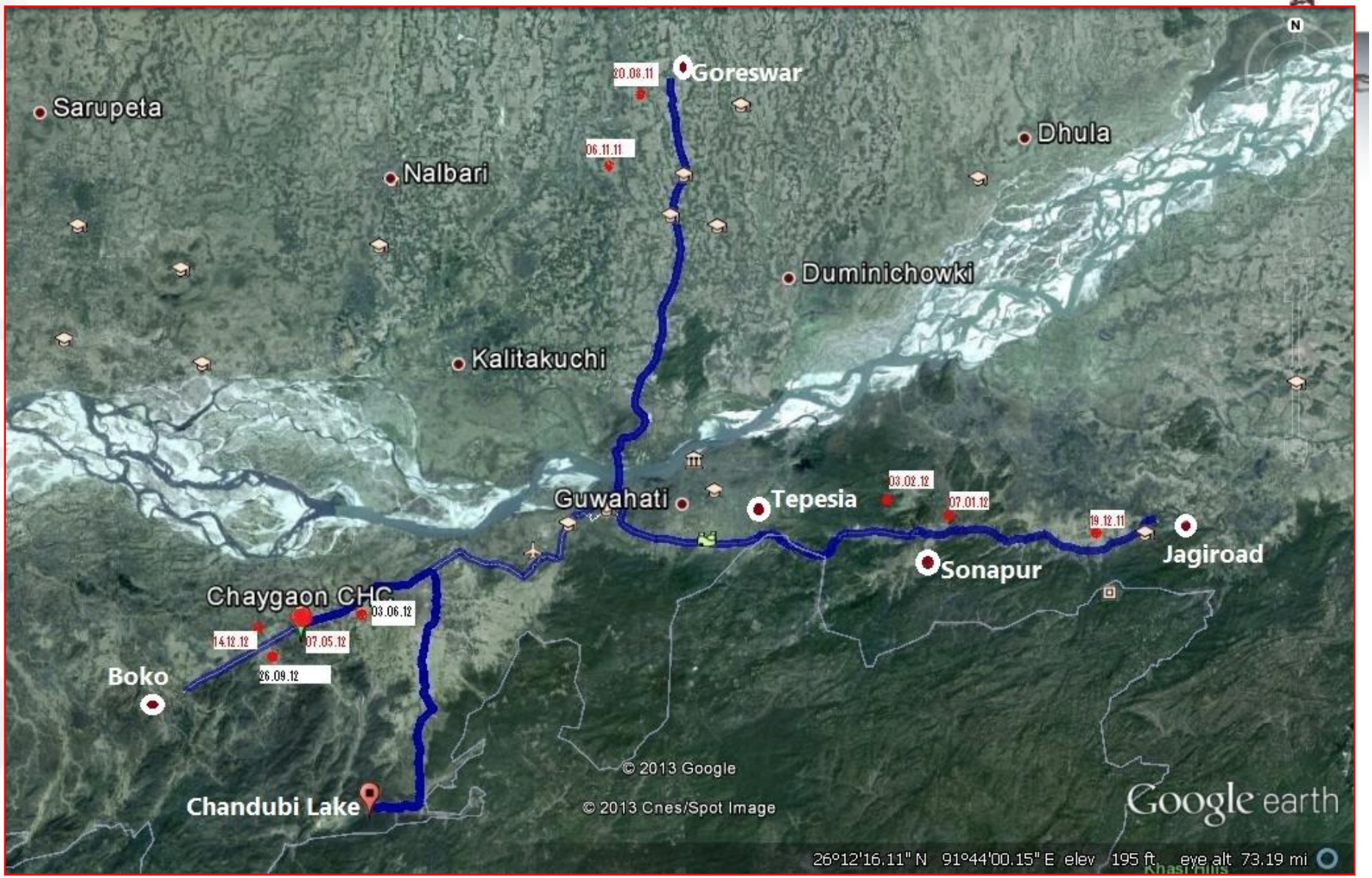
Network analysis of disease spread among farms



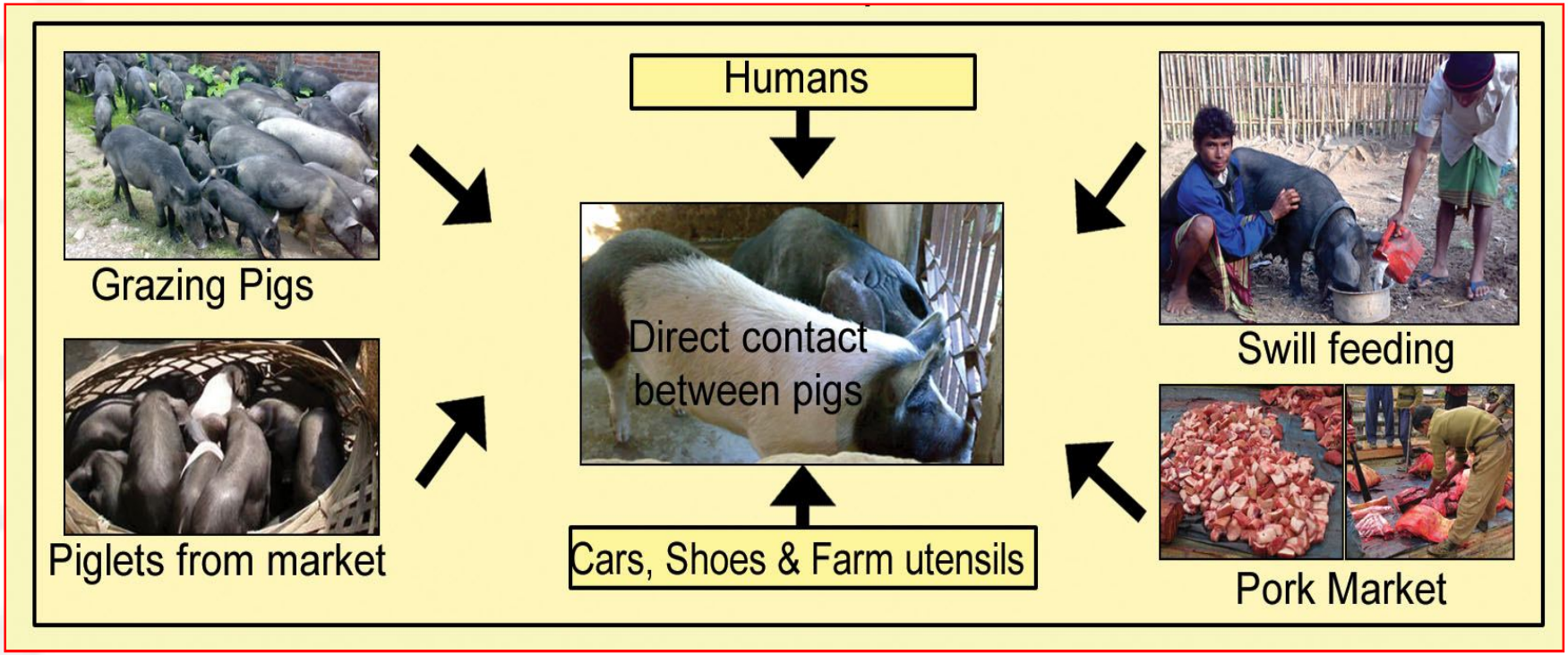
Importance of understanding networks



Tracing of Outbreak

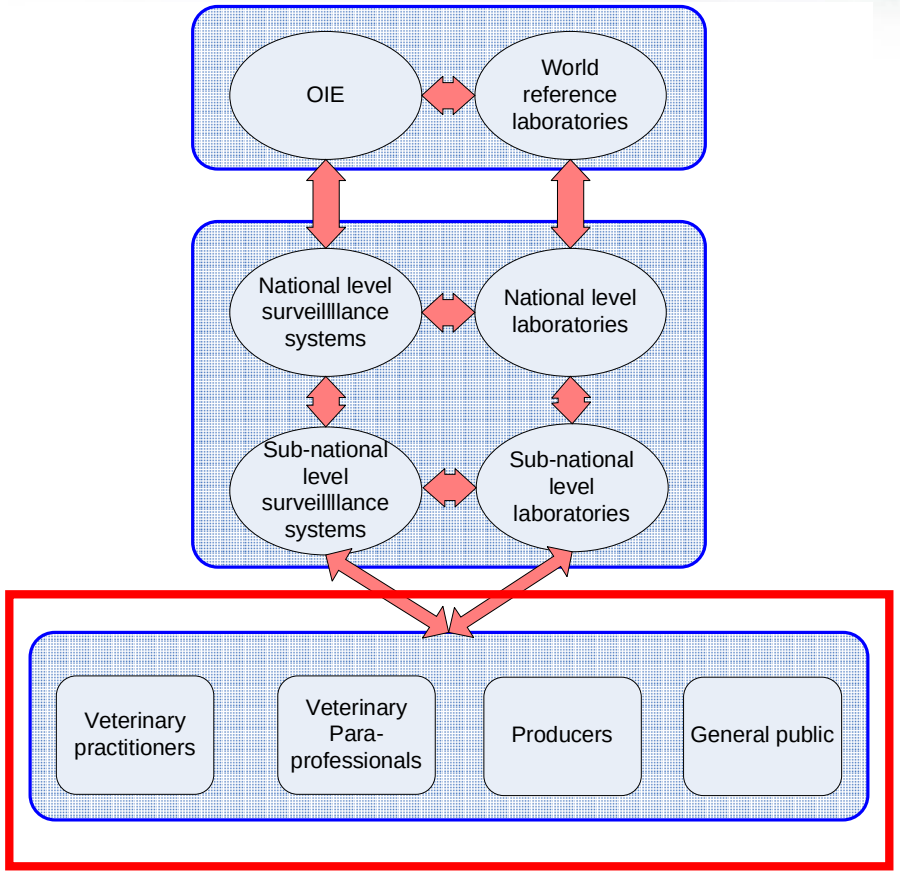


Identifying Transmission Factors



Factors affecting the coverage of surveillance systems

- Geographic coverage
- Awareness of field veterinarians and farmers
  - What to report? To whom? What happens if I do?
- Economic incentives
  - Possible consequences of disease reporting
  - Conflicts of interest
- Compensation
  - Inadequate or inexistent programs



Constraints for disease surveillance

- Lack of sustainable funding for field services;
- Remote, inaccessible areas are common,eg yak
- Government veterinarians - office-bound;
- Govt animal health service low in rural areas;
- Disruption of veterinary service by conflict;
- Lack of infrastructure to support effective surveillance
- Reporting and reluctance by Govt to promote, accept or integrate with private animal health care service delivery.

Disease surveillance: Indian Scenario

- Protection of Animal Health - State Govt,

- Govt of India-Department of Animal Husbandry, Dairying and Fisheries (DADF) - coordination and control of animal diseases at national level.

- Indian Council of Agricultural Research (ICAR) - Animal Science Division - animal disease research, diagnosis and their control.

Disease surveillance and DADF

- ***Disease Diagnostic Laboratories***

- **CDDL -CADRAD**

- **Five RDDL**

  Kolkata (Eastern),
  Pune (Western),
  Jallandhar (Northern),
  Bangalore (Southern)
  Guwahati (North-eastern)

- ***National Animal Disease Reporting System (NADRS):*** **computerized system of animal disease reporting linking each block, district and State HQ to Central Disease Reporting and Monitoring Unit , New Delhi**

- **NIC - 6,350 blocks, 615-620 districts**

- ***Quarantine stations:*** **Delhi, Mumbai, Chennai and Kolkata. -Bangalore and Hyderbad**

Disease surveillance and ICAR

Three institutes

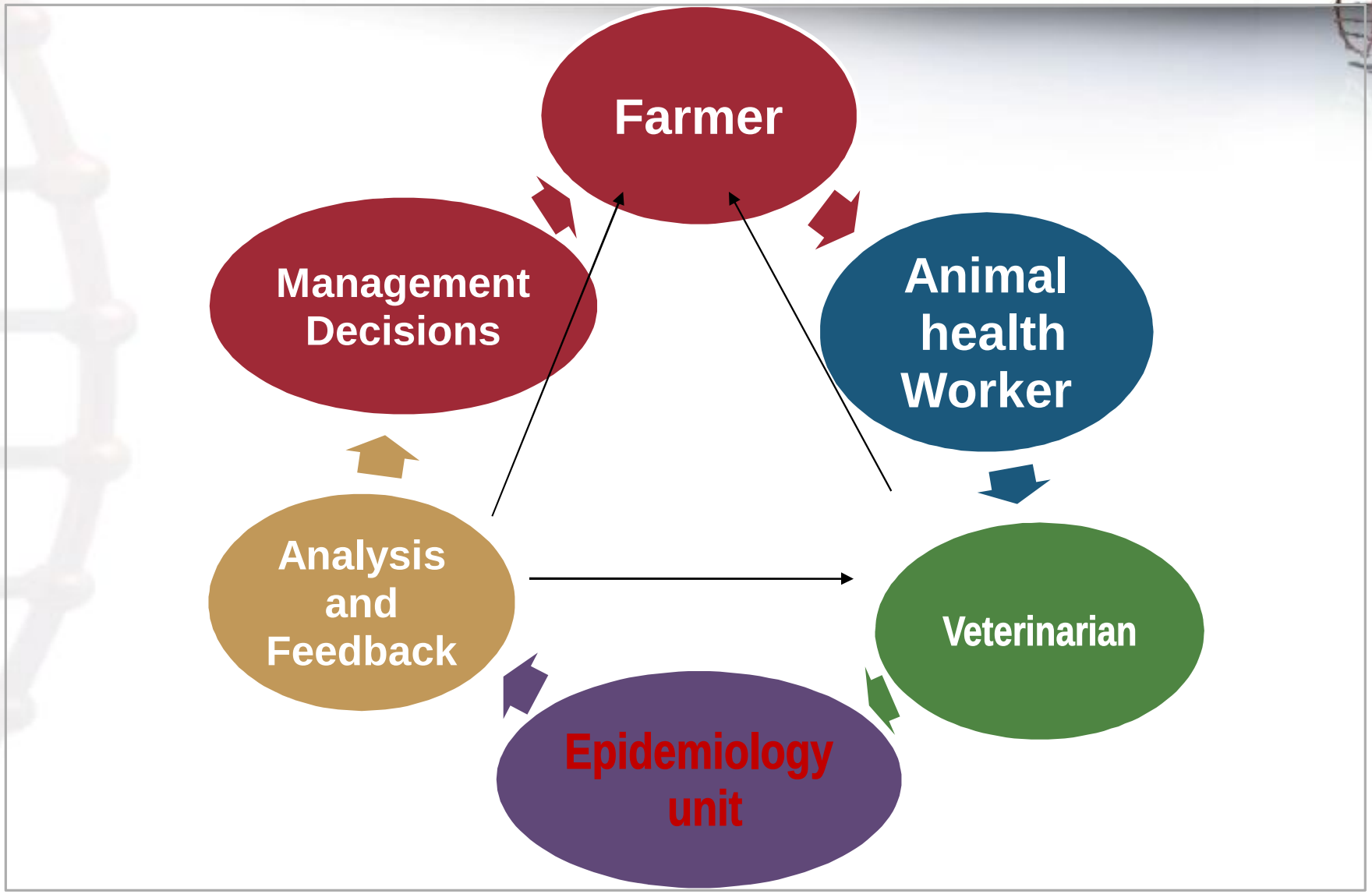
1. Project Directorate on Foot and Mouth Disease (PDFMD)

2. National Institute of Veterinary Epidemiology & Disease Informatics (NIVEDI) earlier (PD_ADMAS)

3. High Security Animal Disease laboratory (HSADL, IVRI)

- *PD_FMD* : FMD
- *HSADL, Bhopal:* Exotic and emerging pathogens - BSL-IV containment laboratory
- *NIVEDI, Bangalore:* Major economically important animal diseases including zoonoses , disease forecasting NADRES (www.nadres.res.in) and diagnostic kits, Disease informatics

Data generation pattern in India



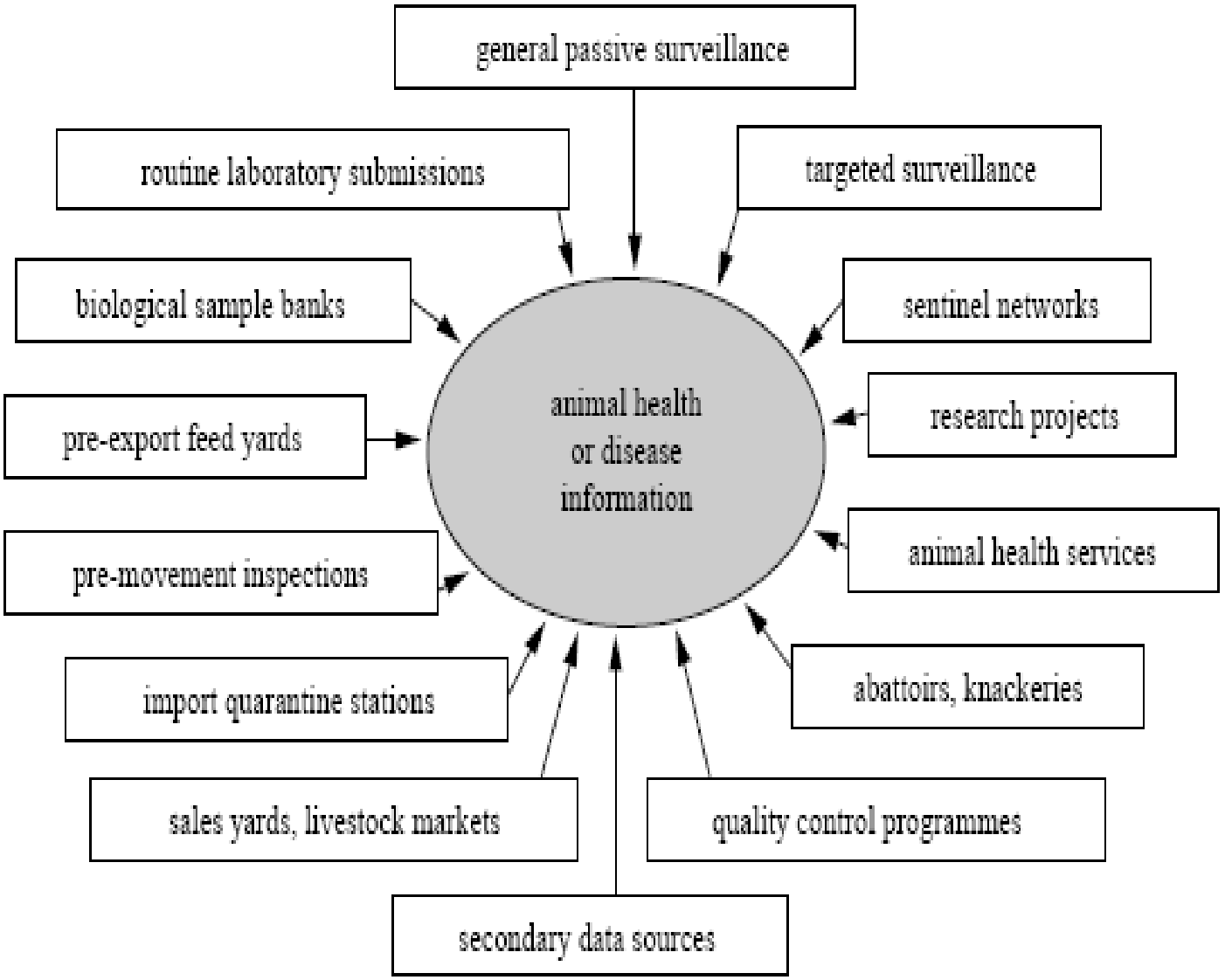
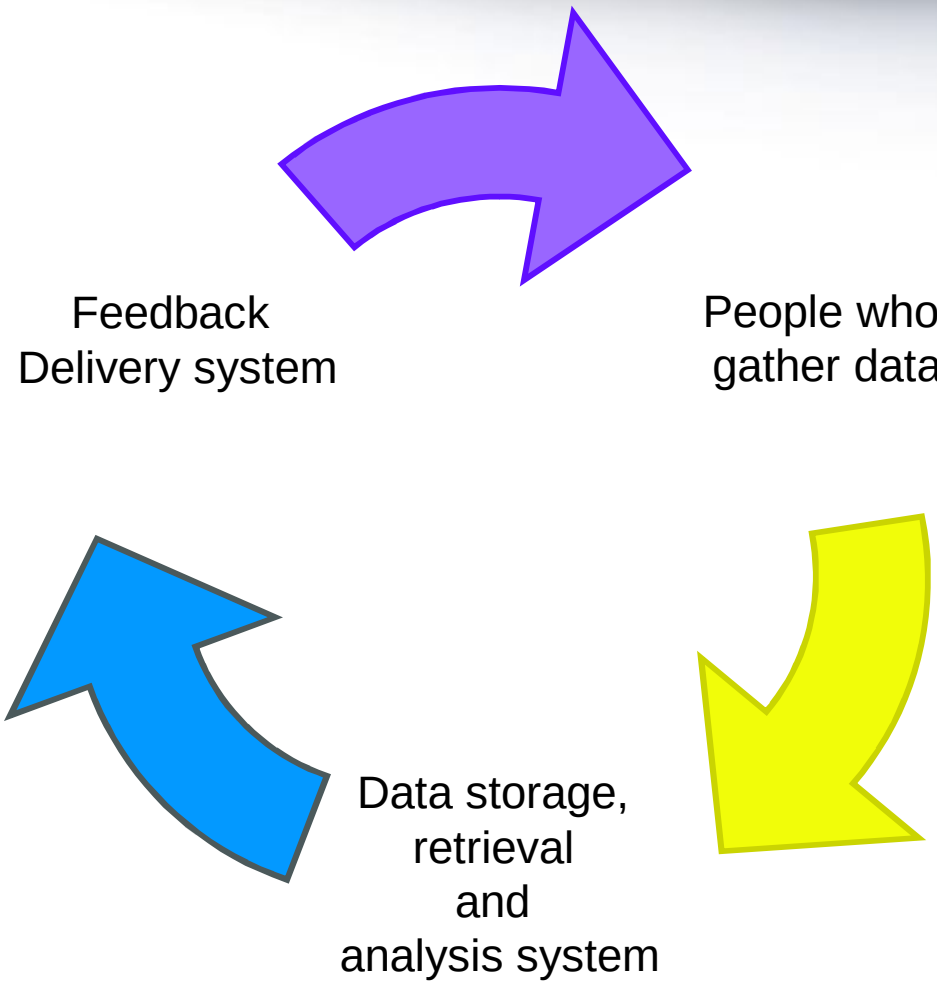


Figure 1. Potential data sources for monitoring or surveillance of animal health-related events.

Disease Recording System



National Disease Database

- Active monitoring through national random sample surveys
- Development of computerized national data bases and networks
- Analysis and GIS mapping of national epidemiological disease data
- Development of interactive national animal health information
- system (AHIS) for the benefit of end-users, contributors, planners, decision-makers and researchers

NIVEDI DATA BANK

Livestock Profile

- Govt. of India conducts livestock census data every five years.
- Although the data is collected at village level, data is compiled at District level.
- This data consists of District level population of cattle, buffalo, sheep, goat, pig, camel, donkey, mule, mithun and yak.

Livestock Disease Profile

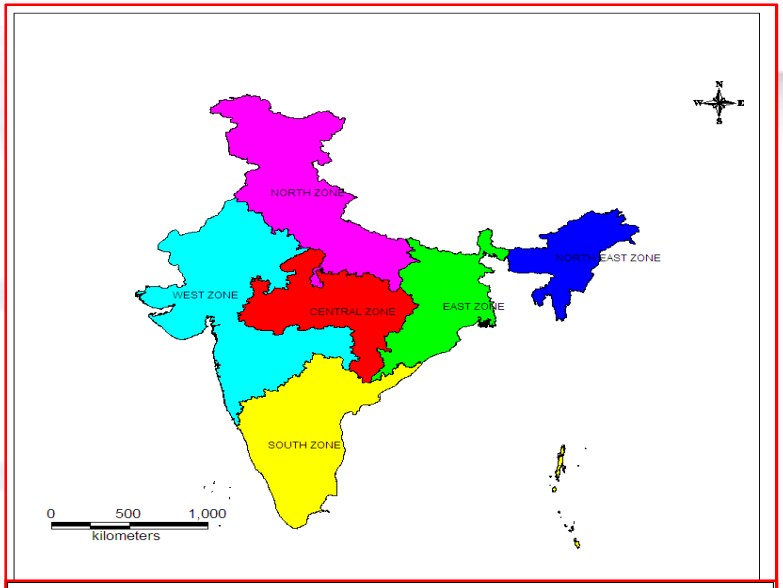
- Data received from the Veterinarians is compiled at **District level** and passed on to **State Directorate** - data on disease occurrence at **State level.**

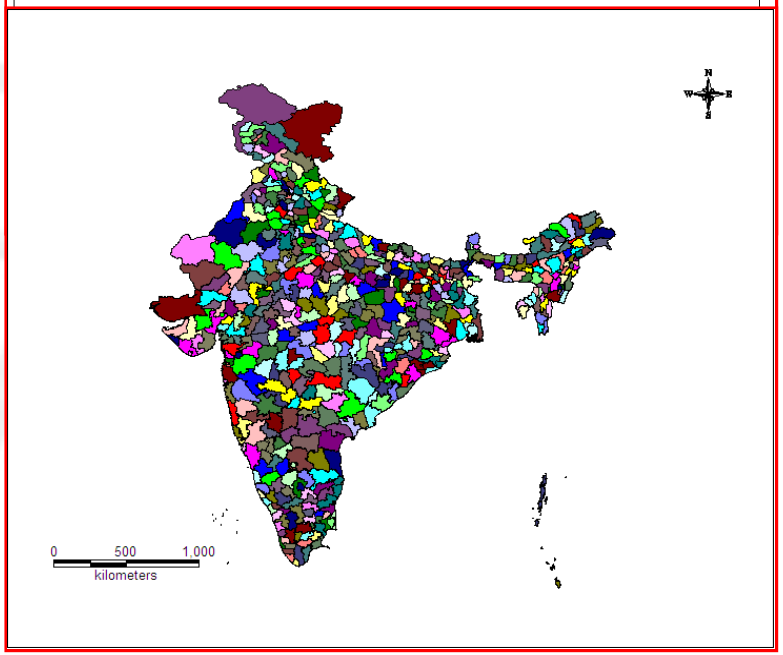
- **District level data is compiled species-wise on monthly basis.**

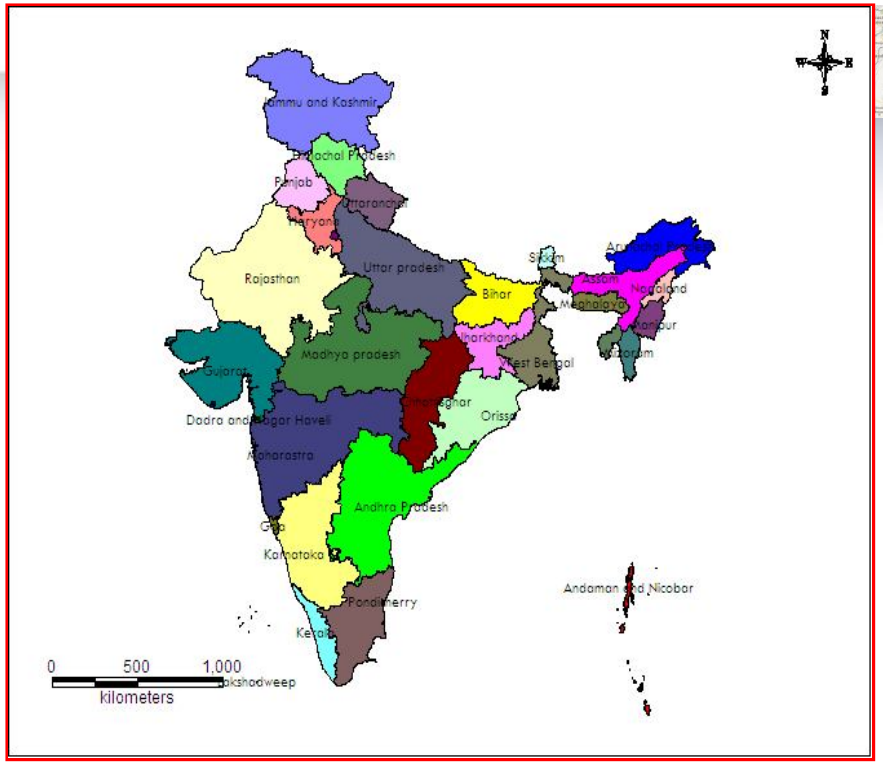
Format of disease data is

1. **Number of Outbreaks**
2. **Number of Attacks**
3. **Number of Deaths**
4. **Number of Vaccination carried out**

Administrative Profile







NIVEDI Data has been divided into

Zones	**6**
States & UT	**35**
Districts	**645**

Meteorological profile

- Extrinsic factors play major role in precipitation of a disease
- Meteorological parameters such as
  temperature, dew point, humidity, sea level pressure, visibility, wind speed, precipitation, etc.

- **81 meteorological stations** across India.
- Stations are grouped **into 44 meteorological zones**

- Data linked to **district profile** and used for **spatial and temporal analysis.**

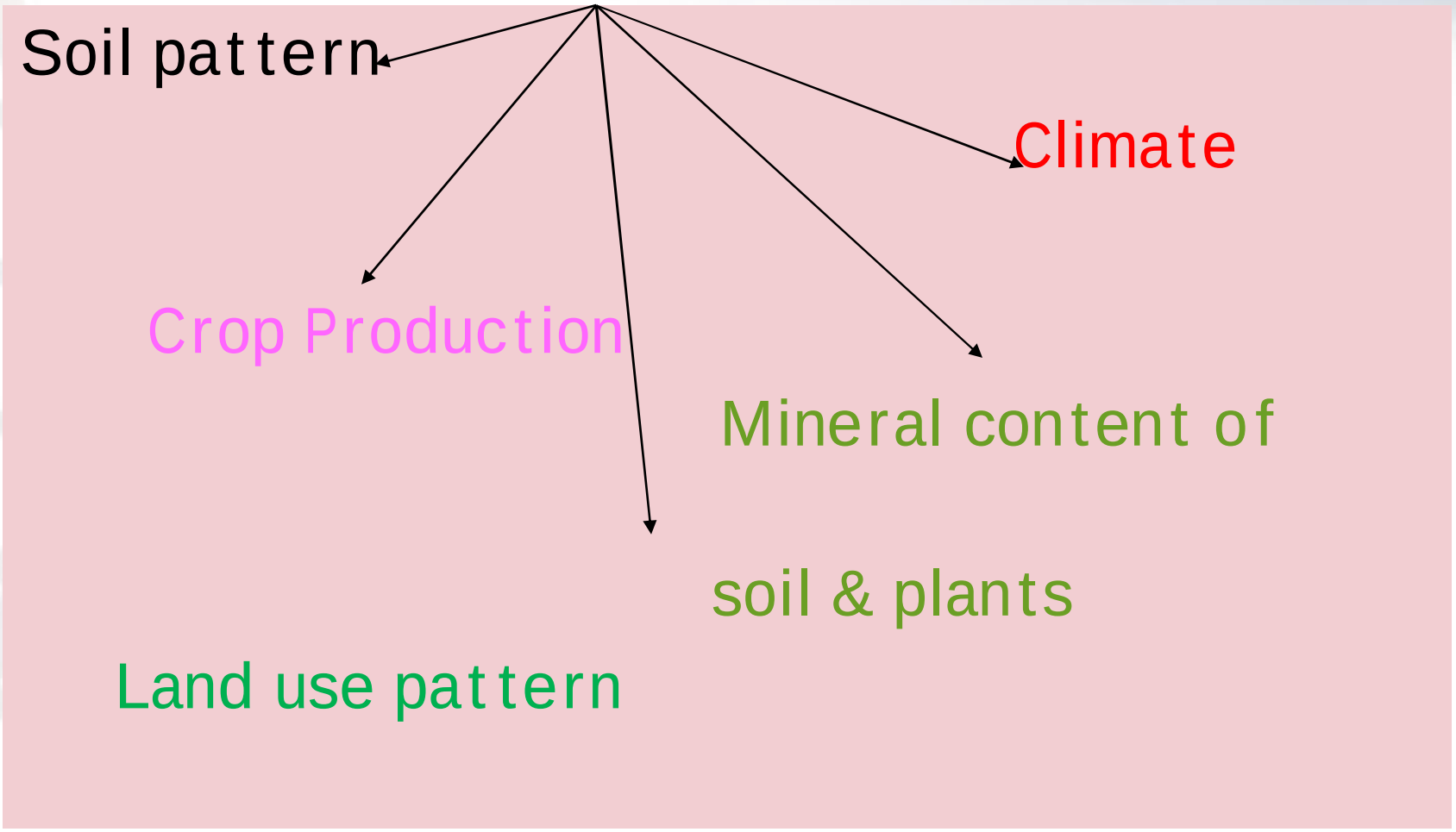
- These **dynamic data** in the databank is **updated regularly** after suitable **validation.**

Agro-climatic Zones of India

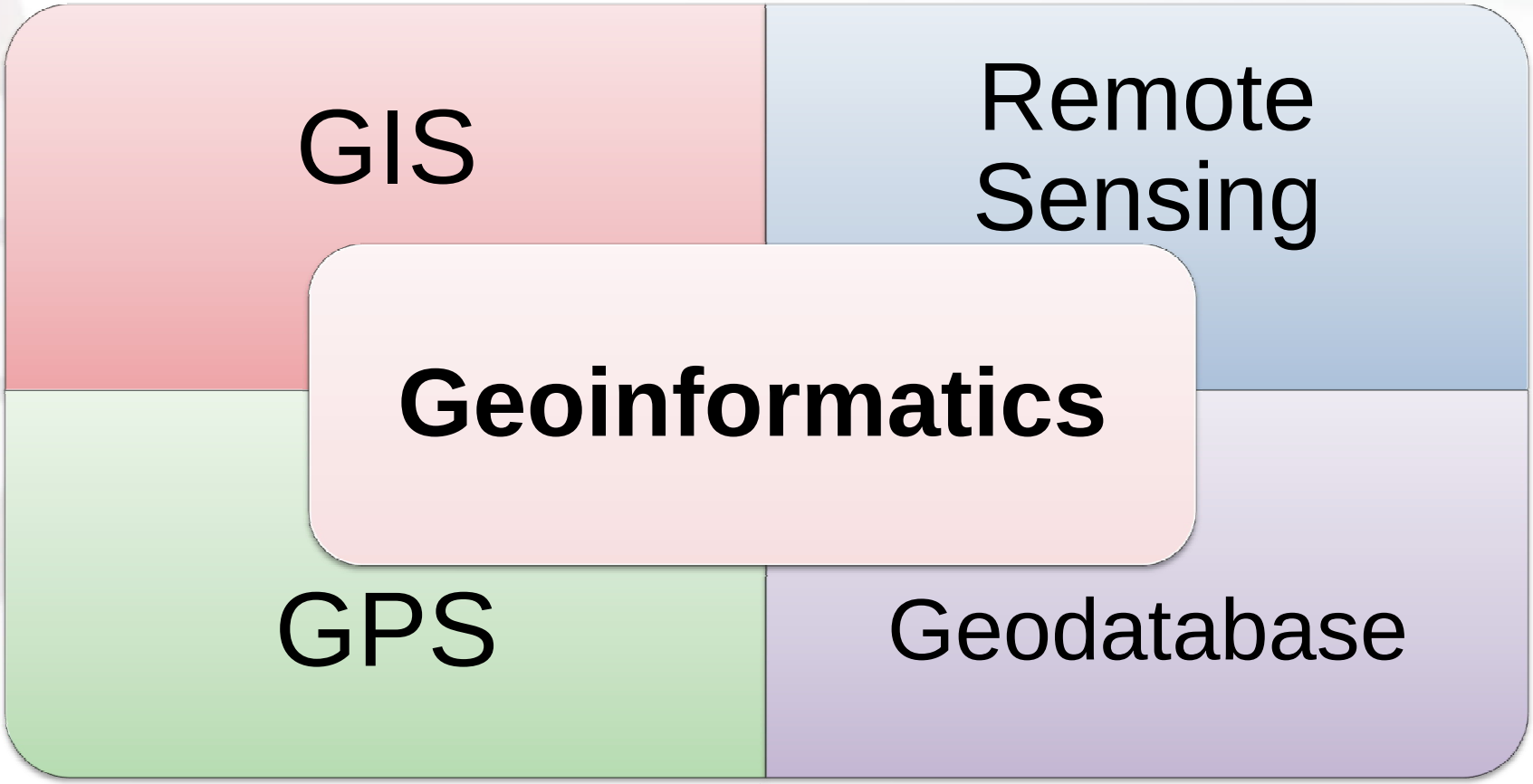
(Unit of land defined in terms of major climate and growing period, which is climatically suitable for certain range of crops and cultivars)

1. Western Himalayas Cold Arid eco-region
2. Western Plains Hot Arid ecosystem
3. Deccan Plateau Hot Arid eco-region
4. Northern Plain and Central Highlands eco-region
5. Central Highlands, Gujarat Plains and Kathiawar, Peninsula eco-region
6. Deccan Plateau, hot semi-arid Eco-Region
7. Deccan (Telangana) Plateau and eastern Ghats, hot arid eco-region
8. Eastern Ghats, TN Uplands and Deccan (Karnataka) Plateau, hot, semi-arid Eco-Region
9. Northern, plain hot subhumid (dry) eco-region
10. Central Highlands and Eastern Satpura Range), hot, subhumid (dry/moist) eco-region
11. Chattisgarh/Mahanadi Basin Agro eco-region
12. Eastern (Chhotanagpur) Plateau and Eastern Ghats, hot sub humid eco-region
13. Eastern Plain, hot sub humid (moist) eco-region
14. Western Himalayas, warm, moist semiarid to dry sub humid eco-region
15. Bengal and Assam Plain, hot sub humid (moist) to humid (inclusion of per humid) eco-region
16. Eastern Himalayas, warm per humid ecoregion
17. North Eastern Hills (Purvachal), warm, perhumid eco-region
18. Easten Coastal Plain, hot sub humid to semi-arid eco-region with coastal alluvium derived soils
19. Western Ghats and Coastal Plain, hot, humid-per humid eco-region
20. Islands of Andaman-Nicobar and Lakshadweep hot, humid to per humid Islands eco-region

Major Ecological factors that influence the disease occurrence



Geographic information System (GIS)

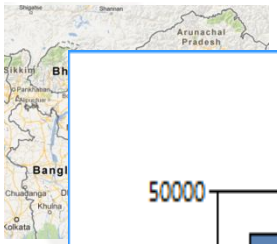


Geographic information System (GIS)

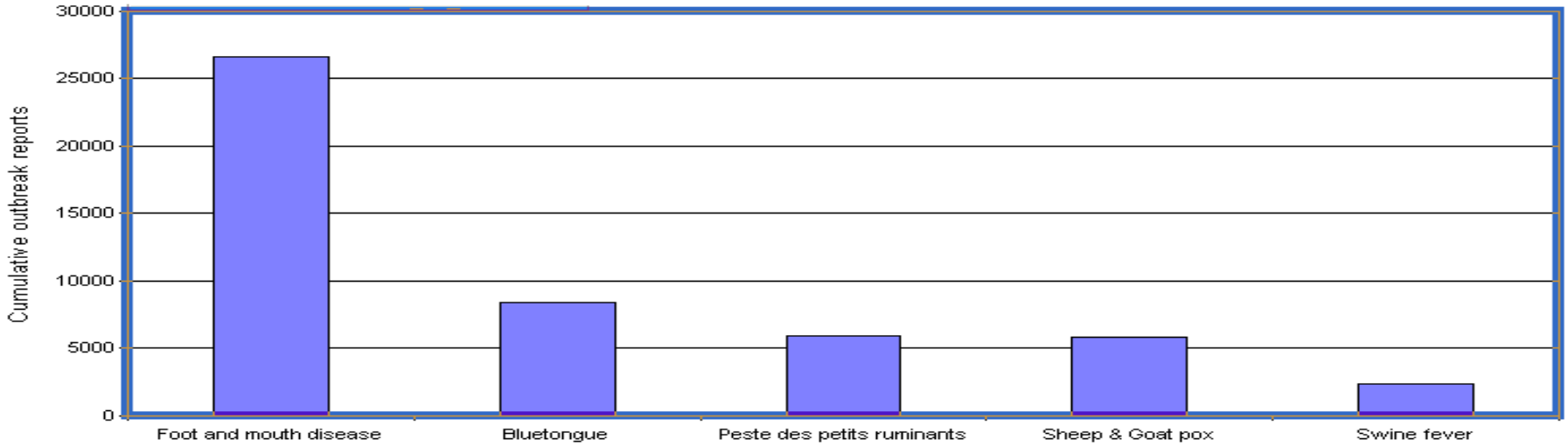
- **GIS** is an **automated system** for the **input**, **storage, analysis** and **output of spatial** information.

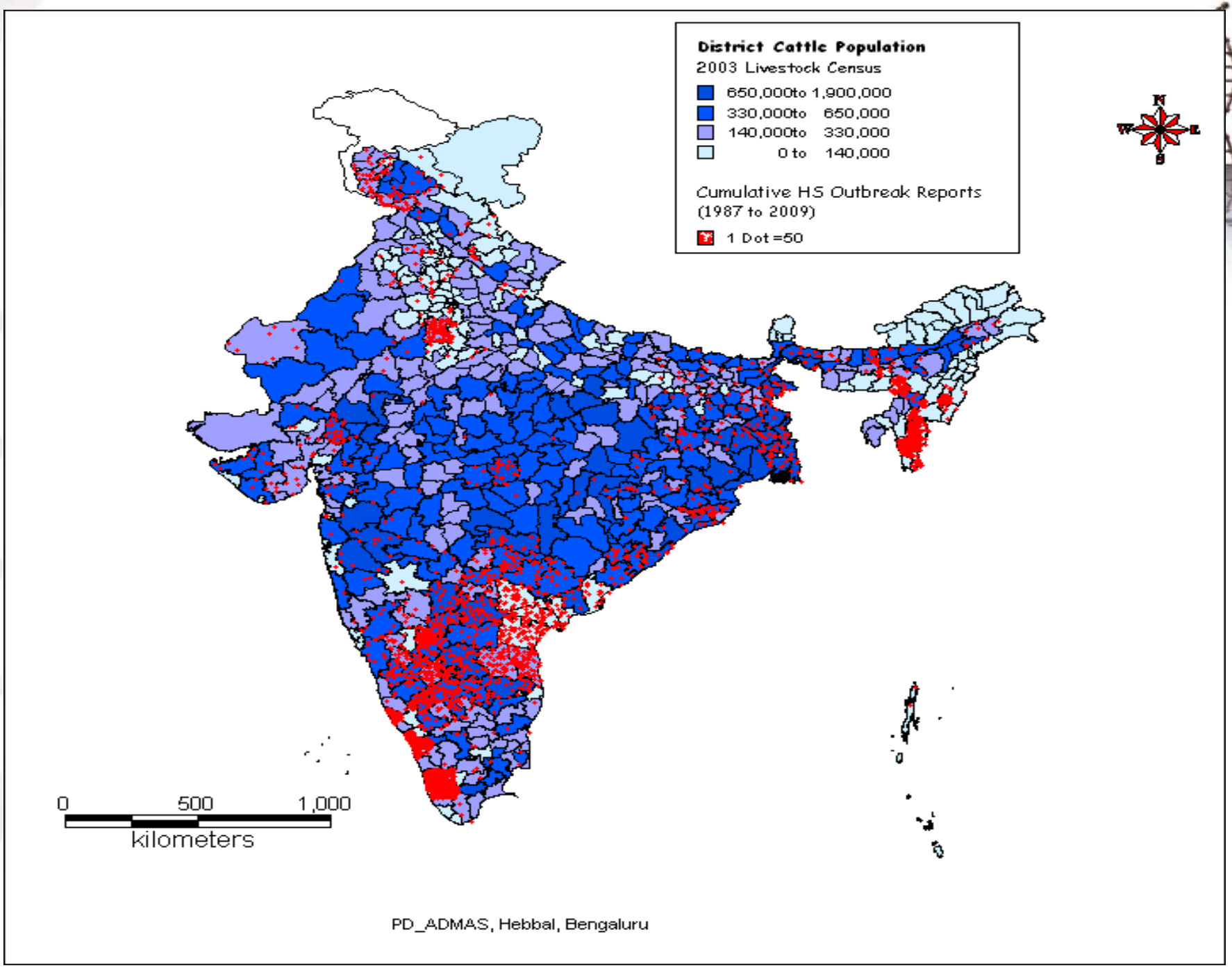
- Various **software packages** are available.
- All are expensive though indispensable.

- **NIVEDI - MapInfo, KIMS and EpiInfo**.

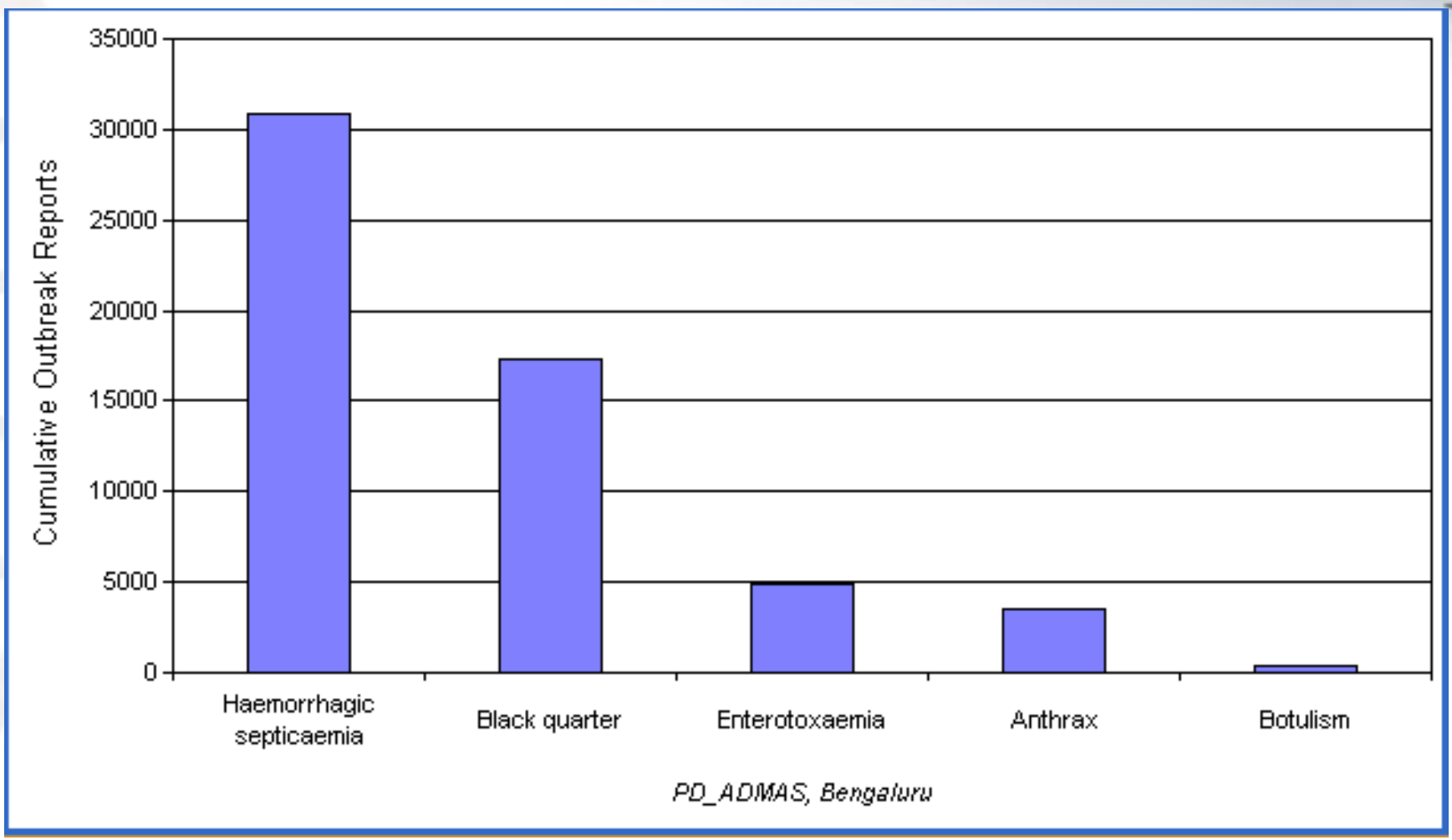


RANKING OF VIRAL DISEASE
(Cumulative OB reports : 1987 – 2011)

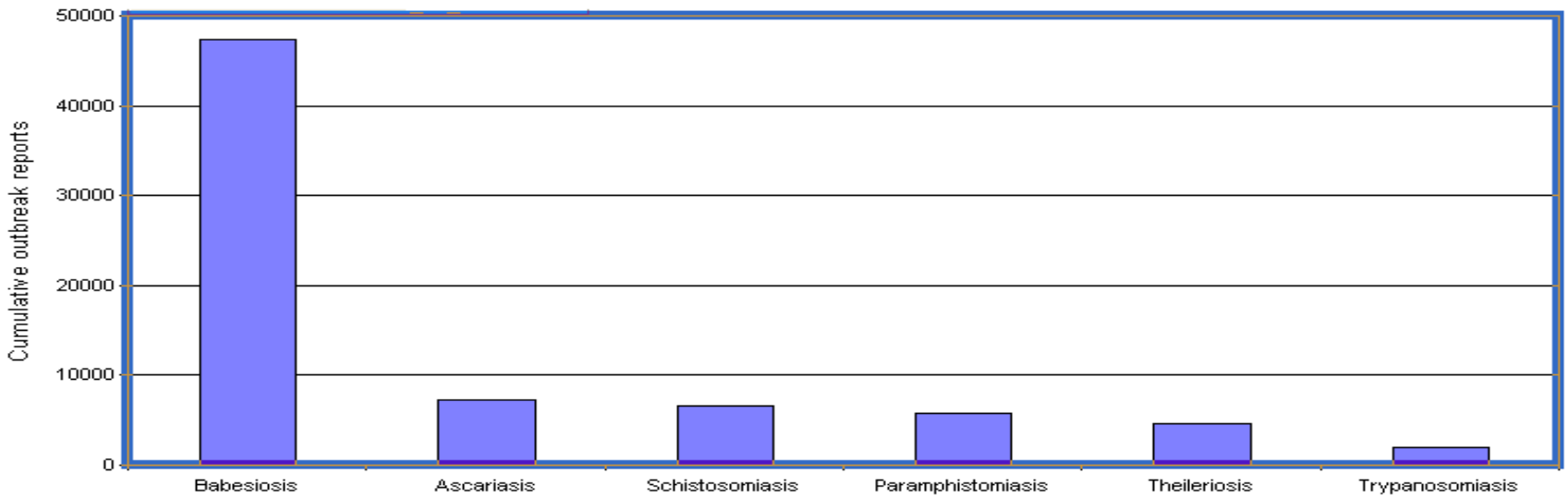


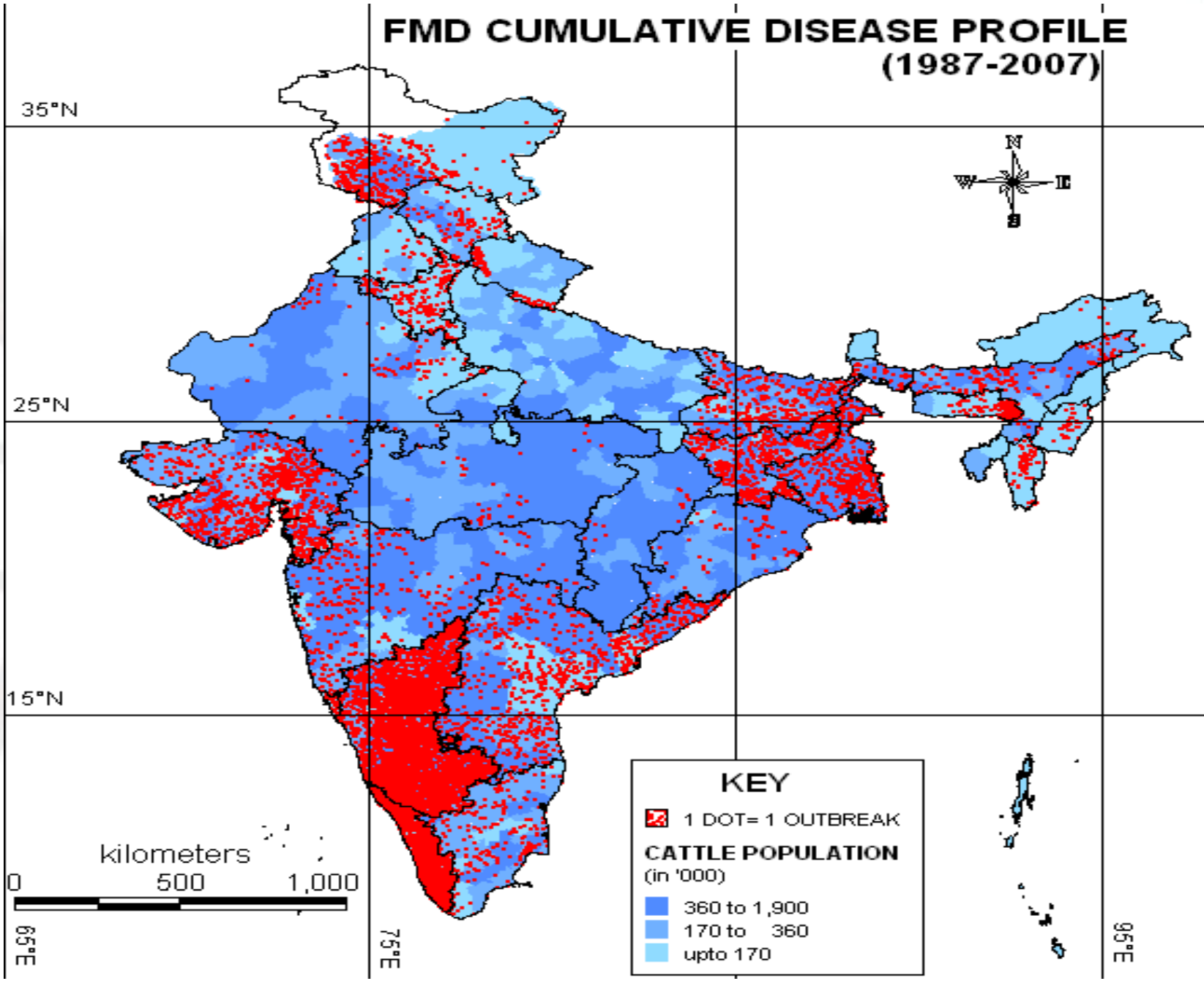


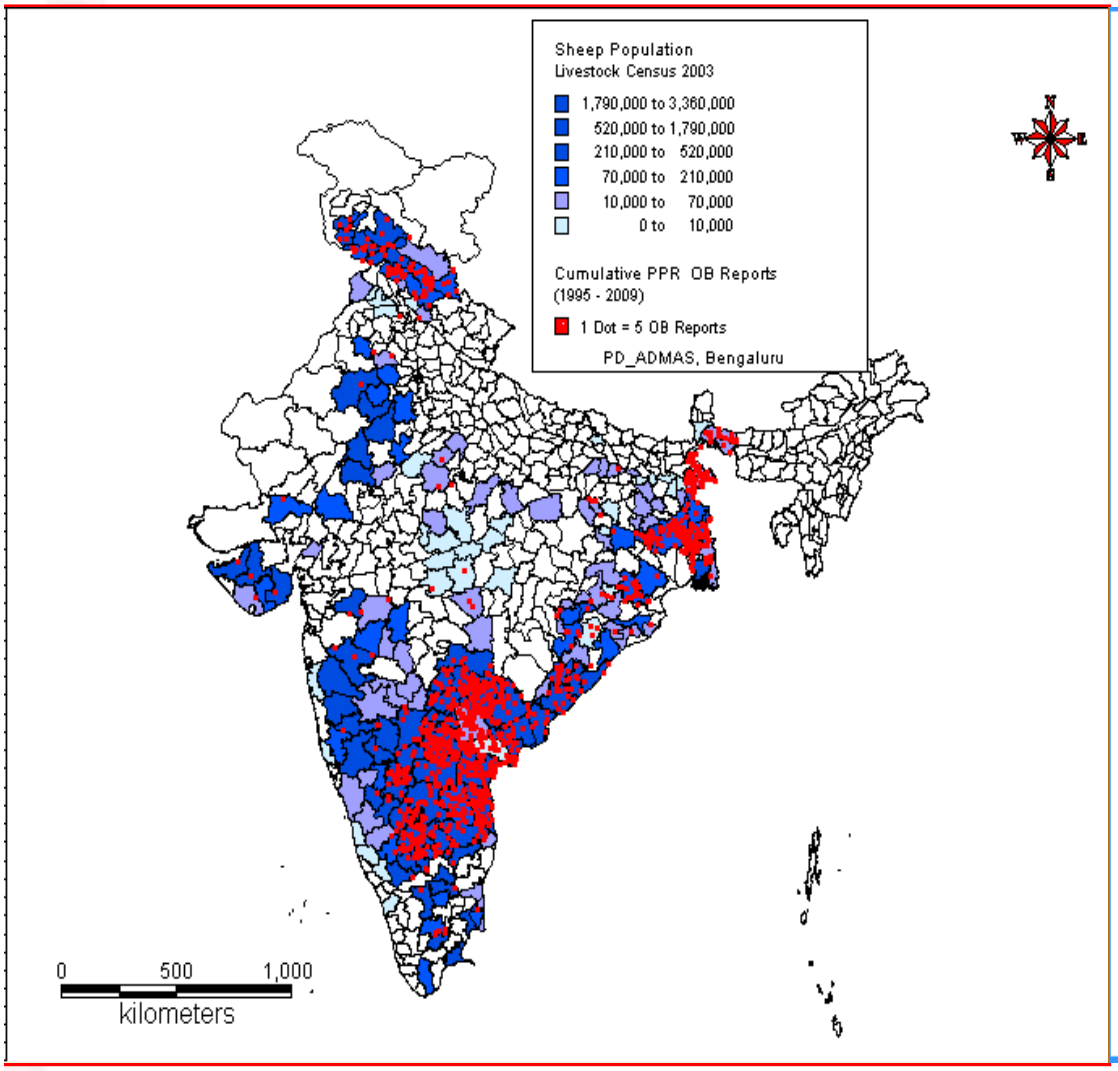
RANKING OF BACTERIAL DISEASE

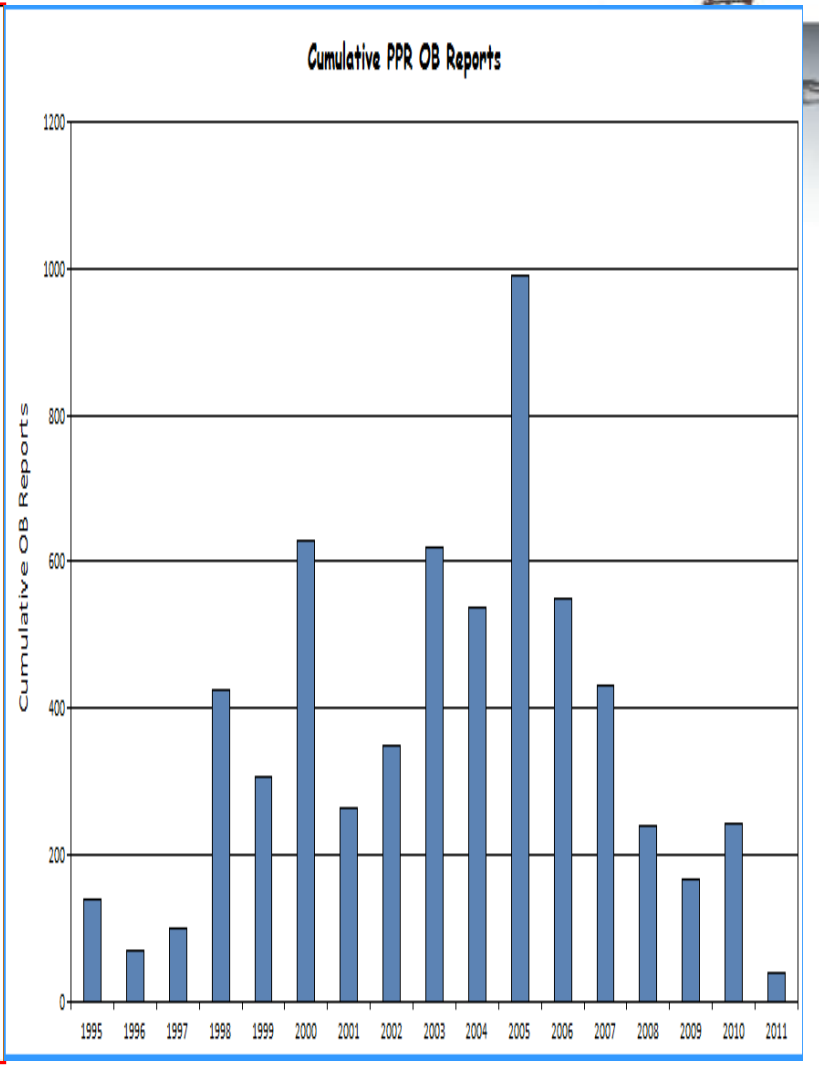


RANKING OF PARASITIC DISEASE

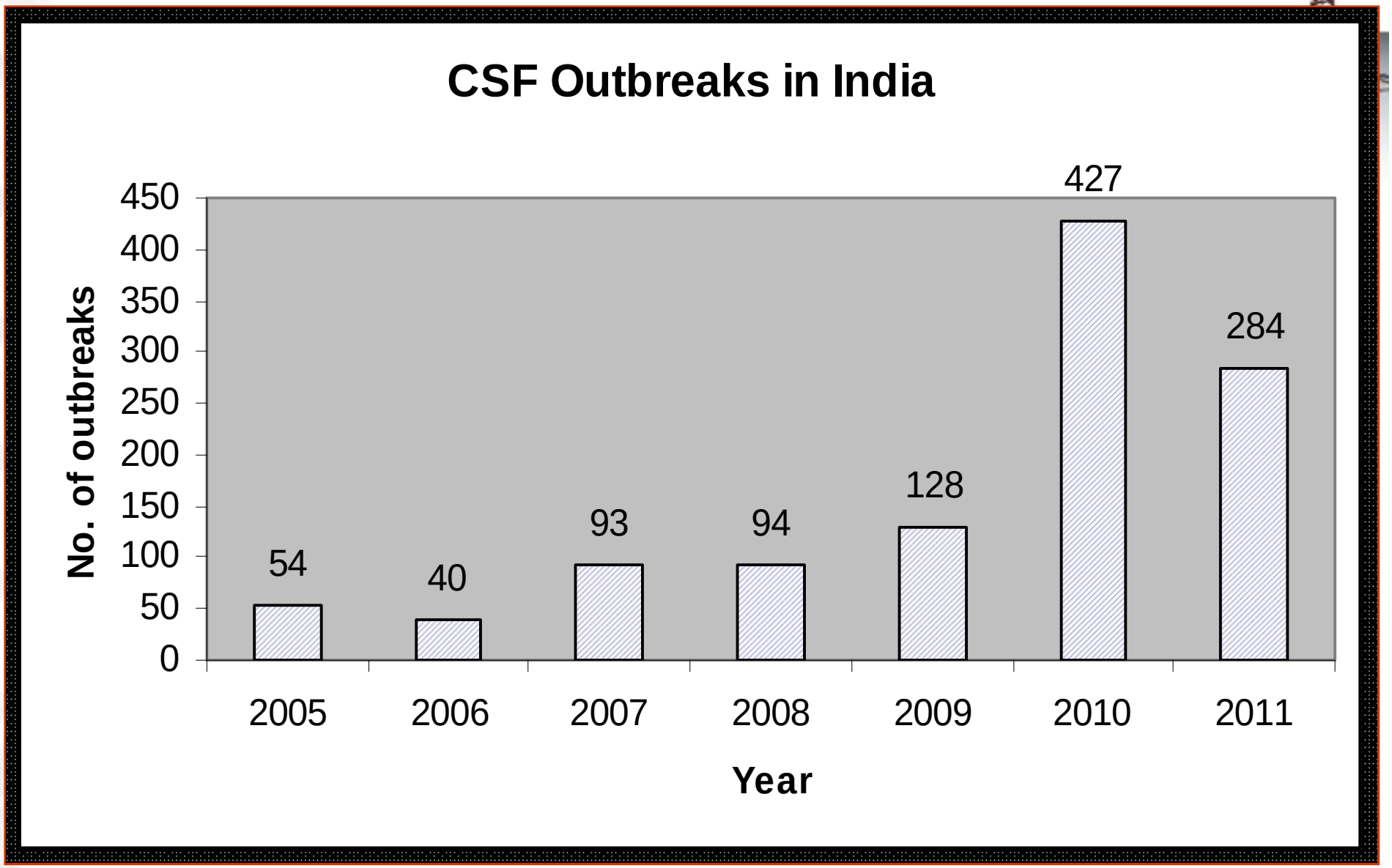




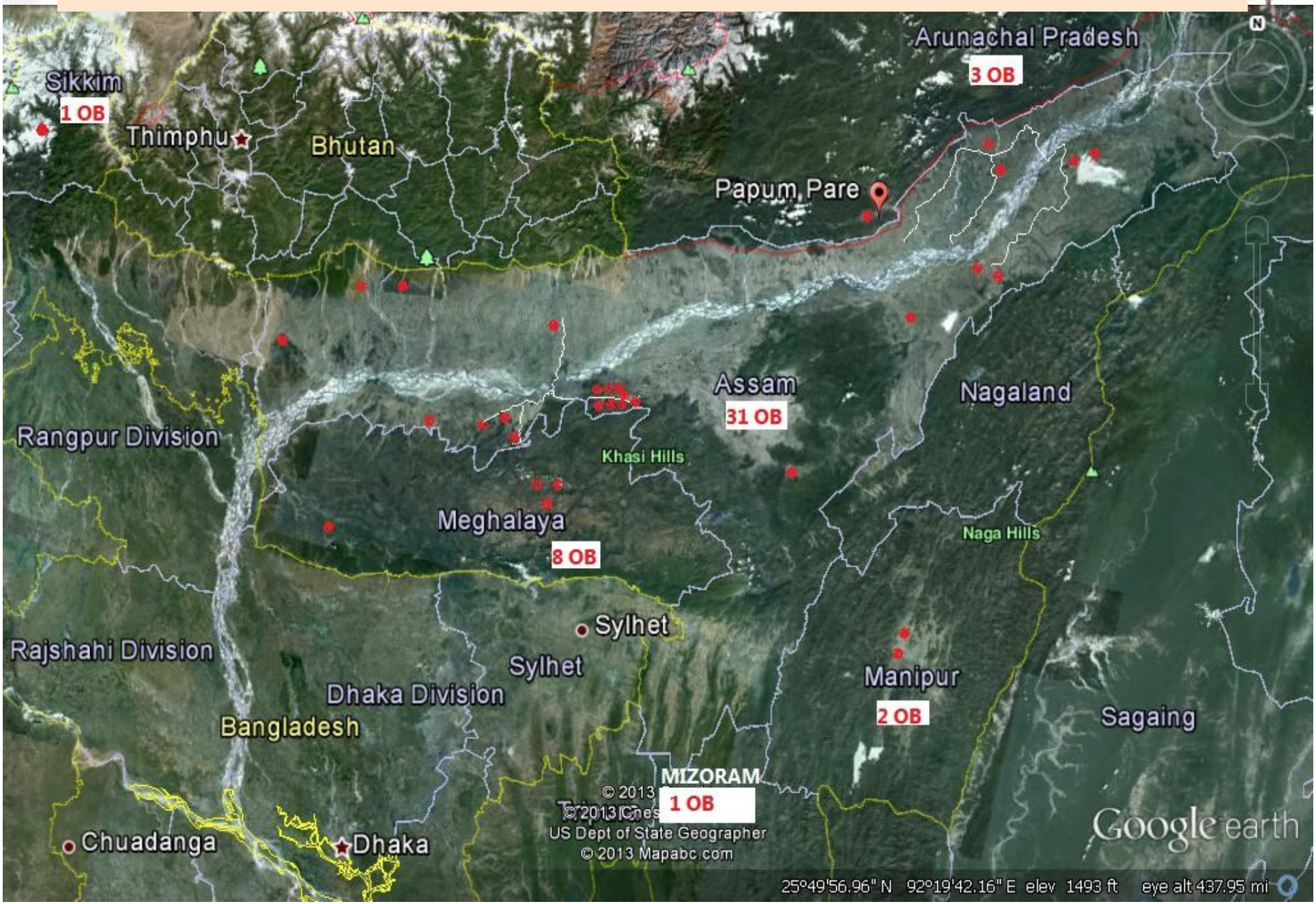


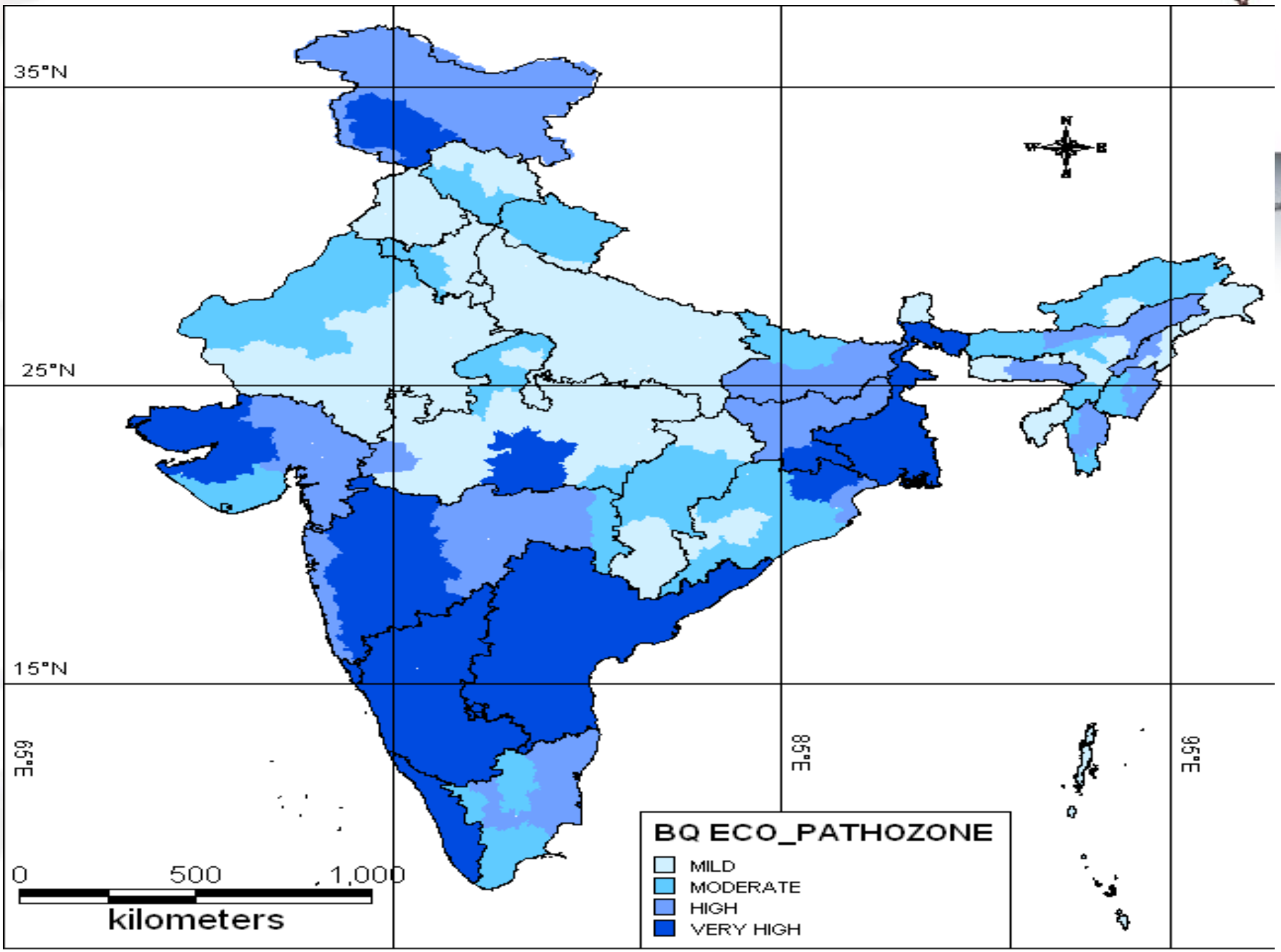


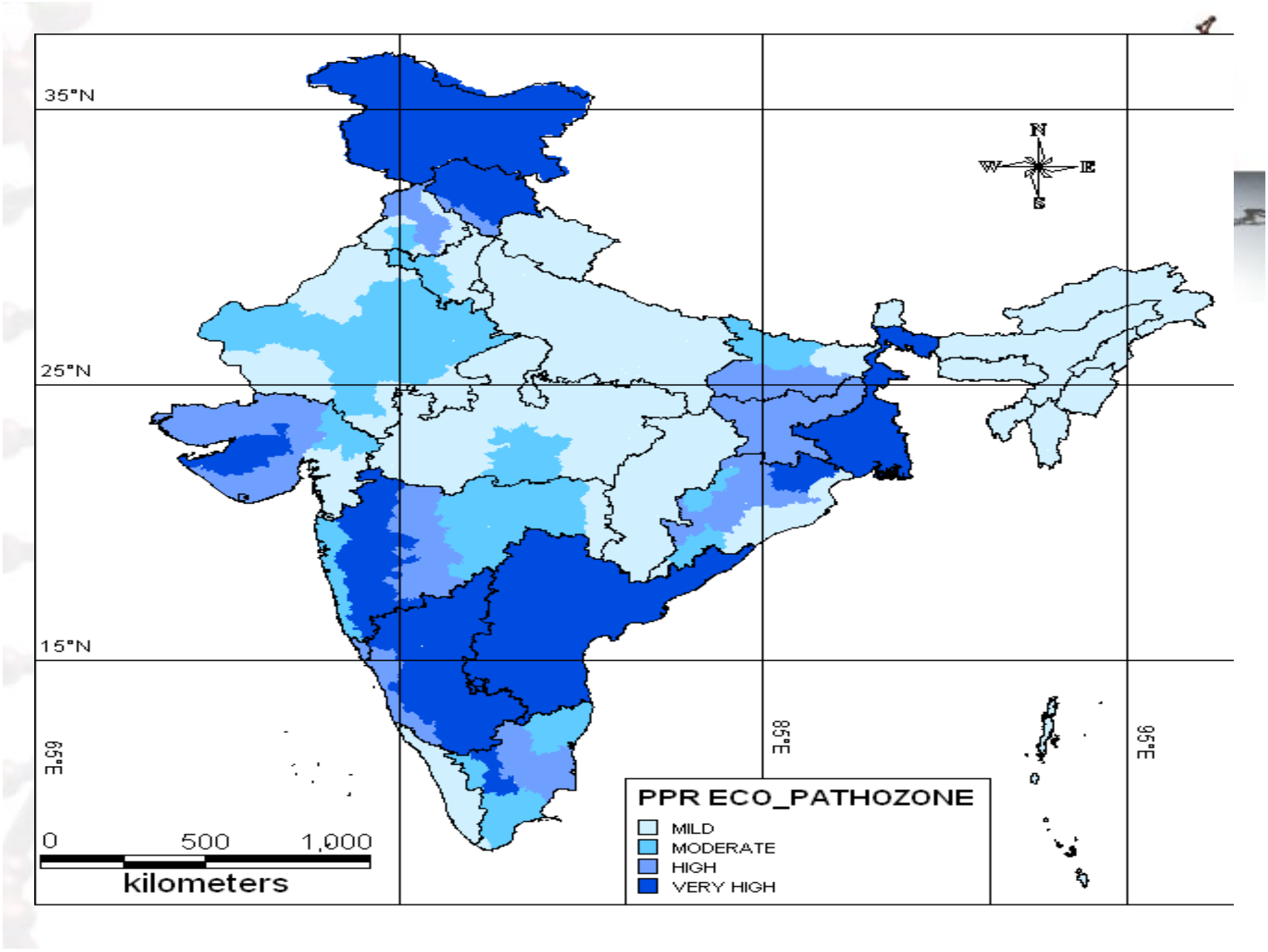
CSF Reported from India

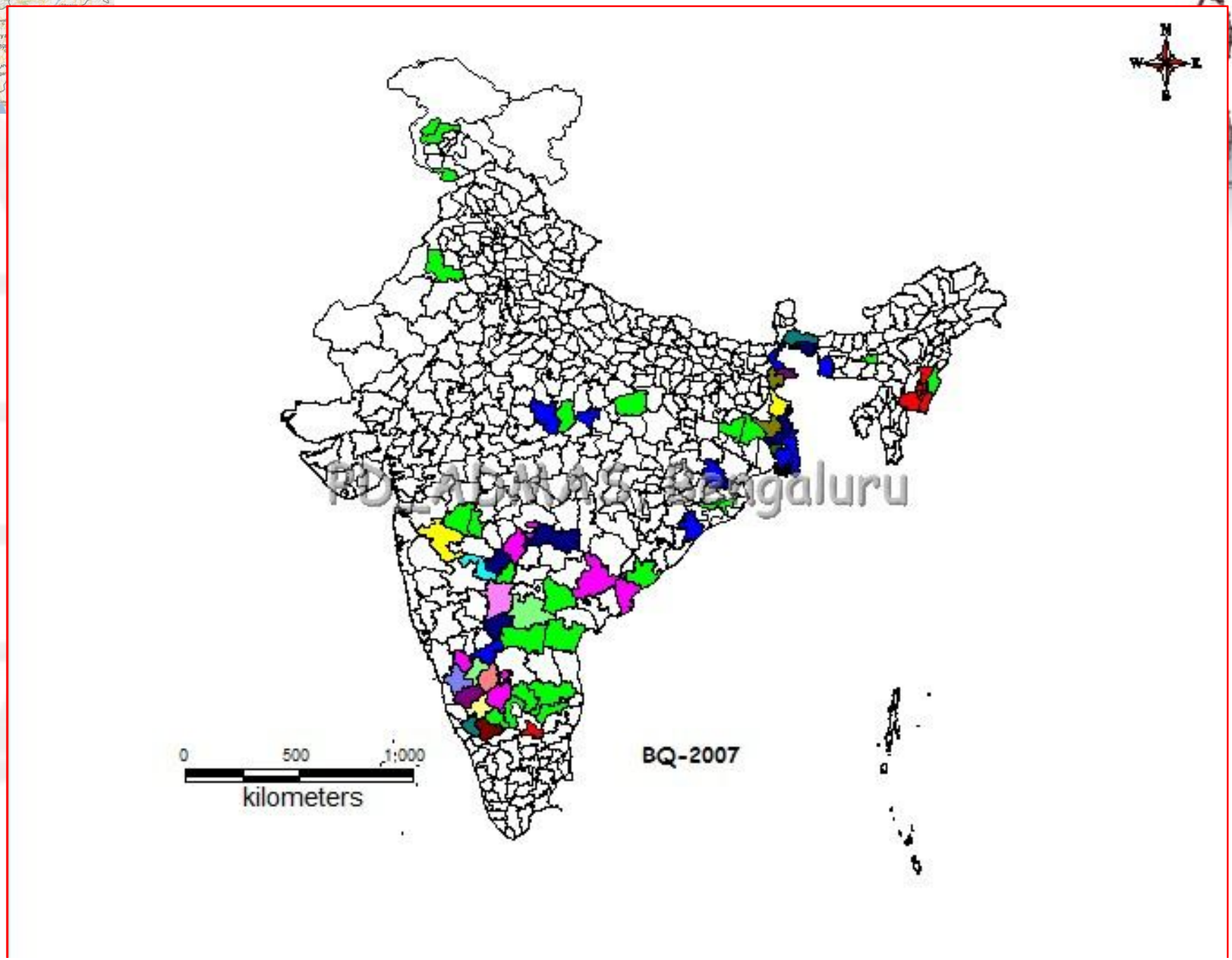


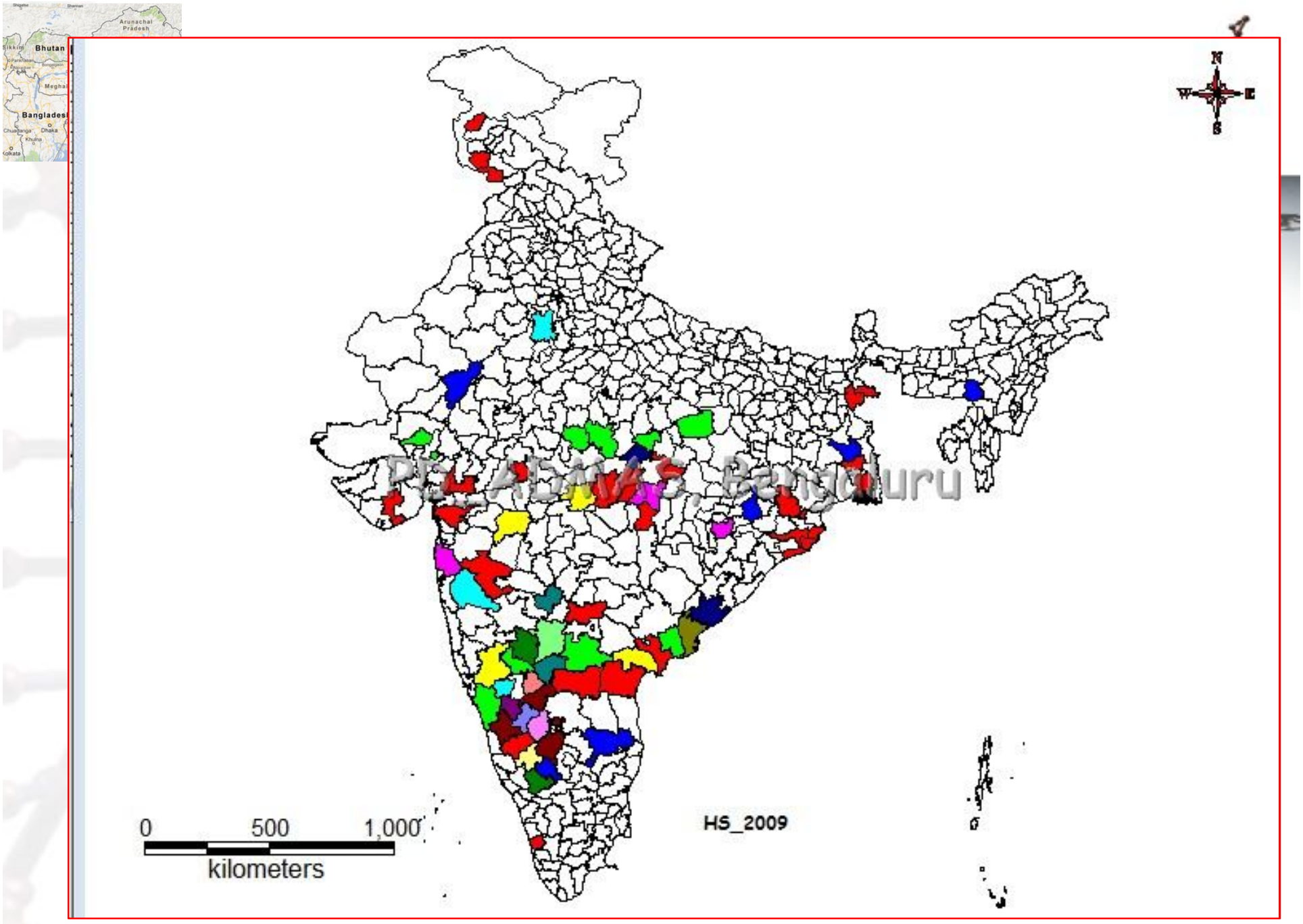
Prevalence of Disease in The NER





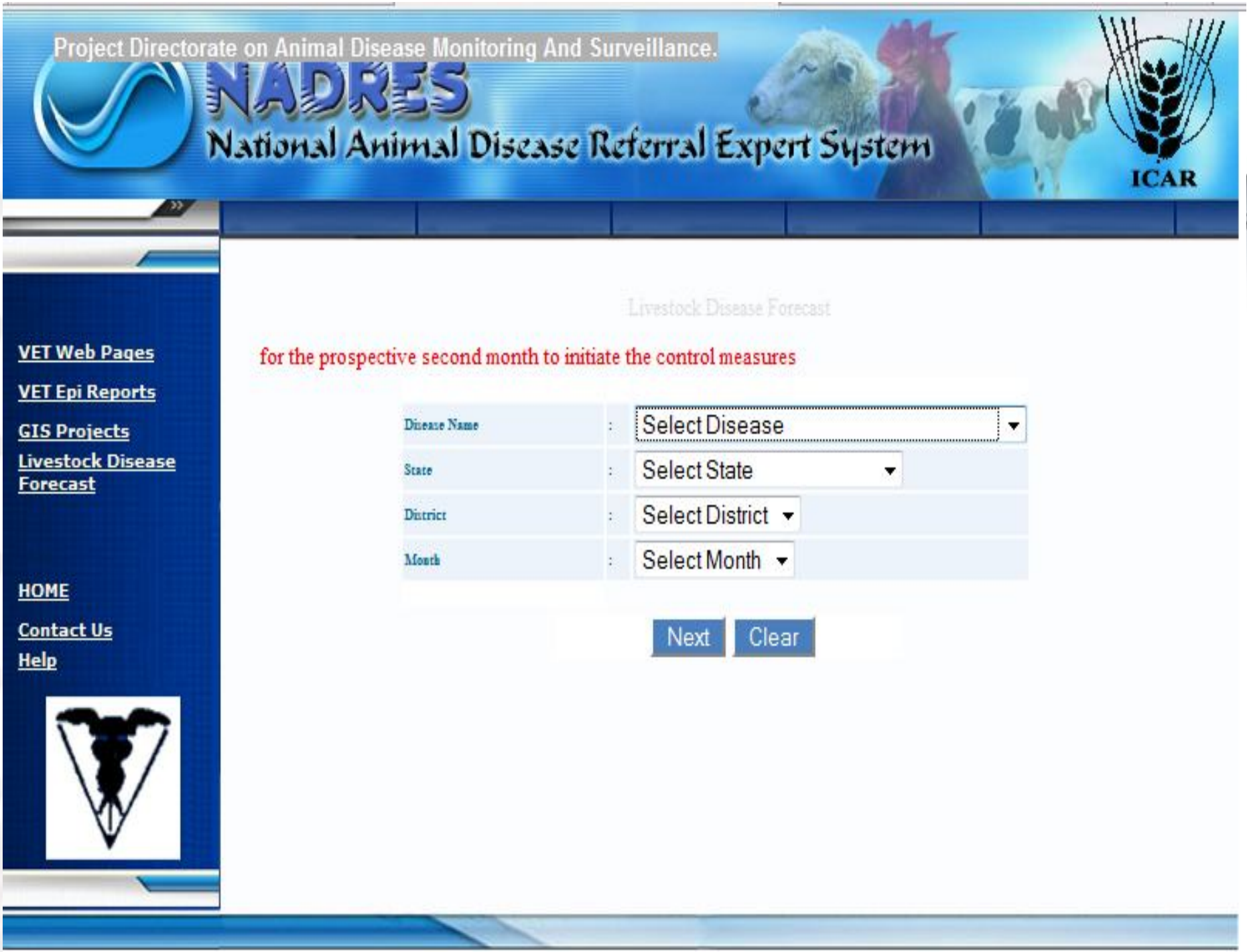


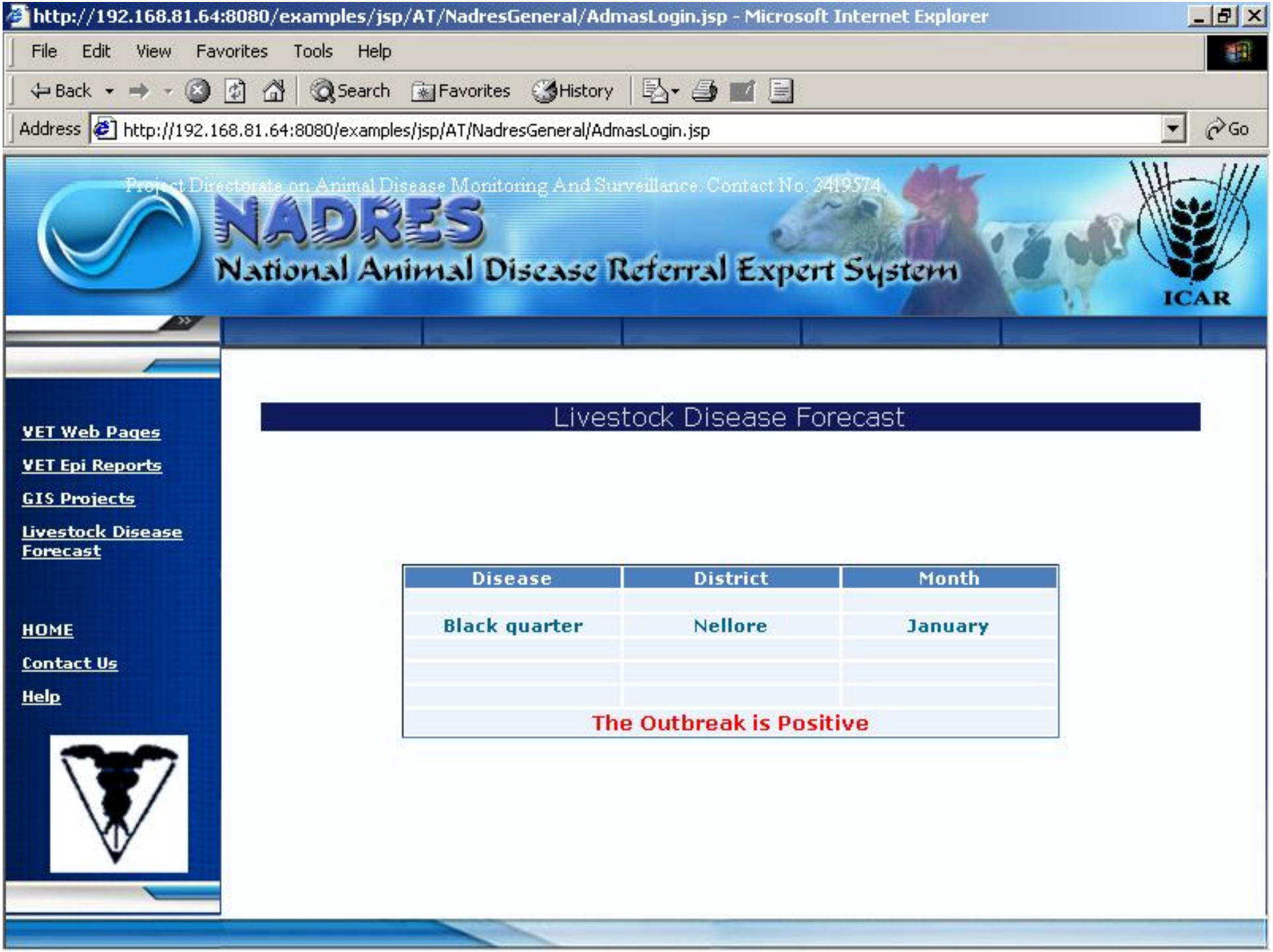


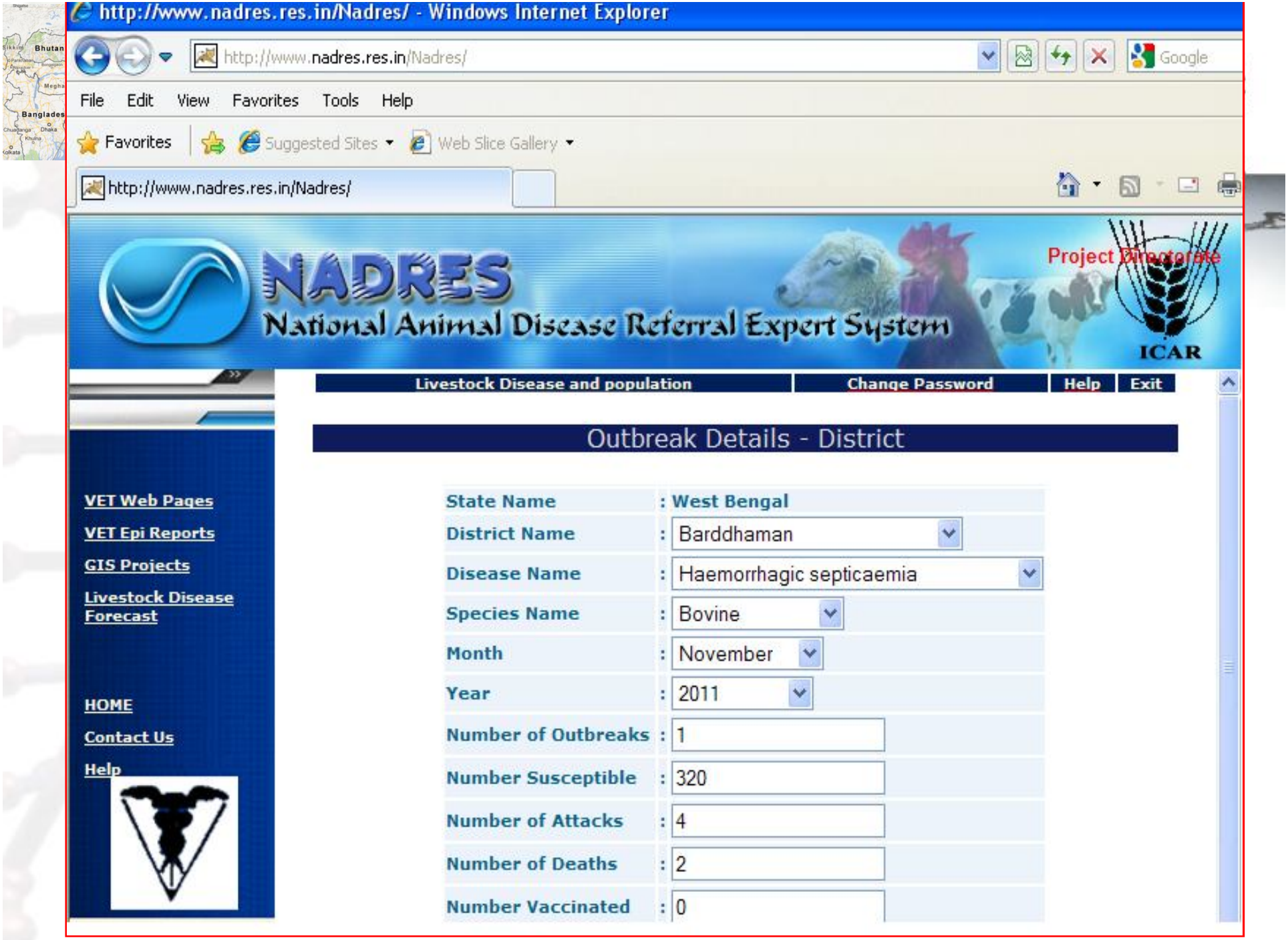


Disease Forecast

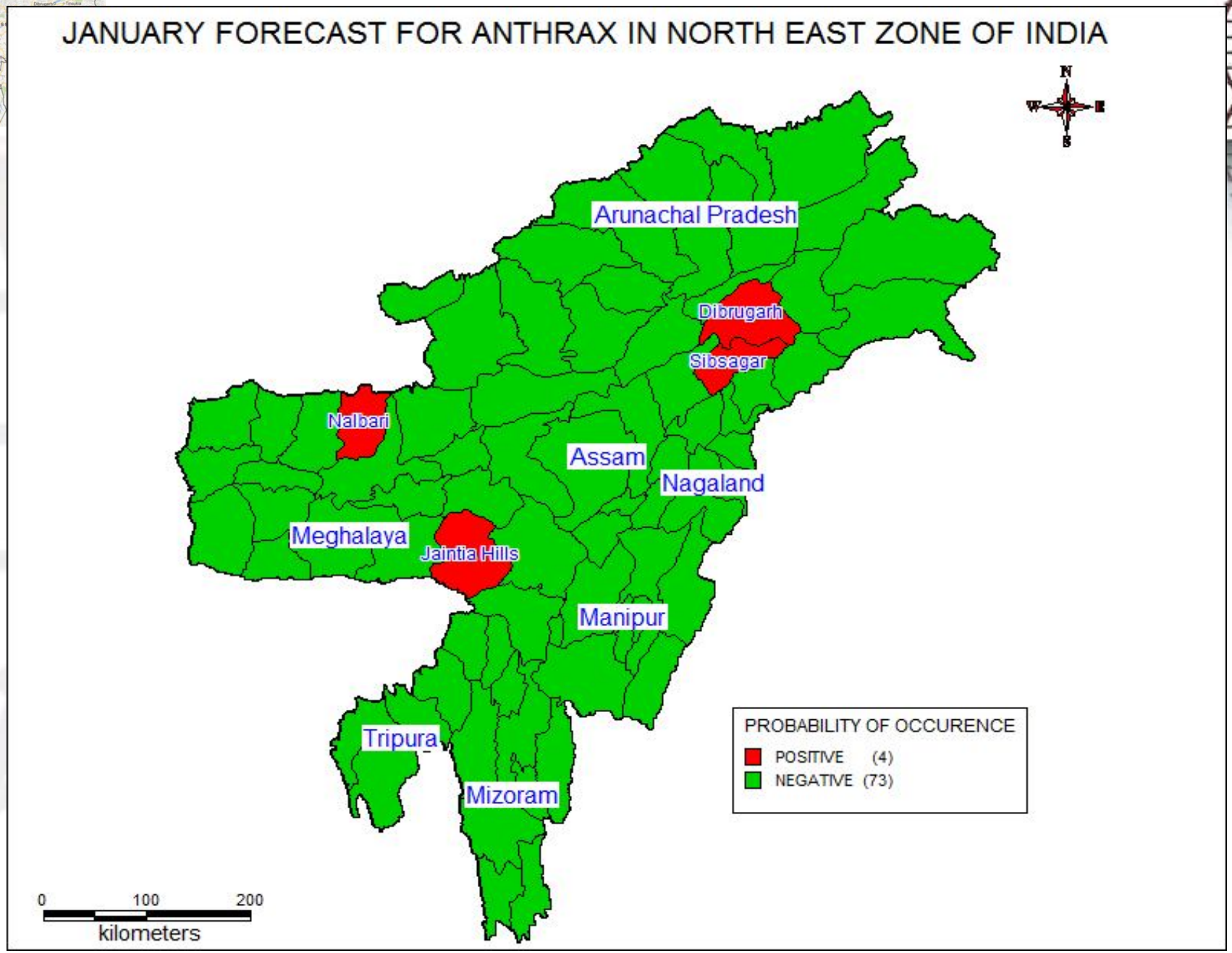
- NIVEDI has utilized this data base and through statistical analysis has developed **National Animal Disease Referral Expert System** (NADRES) a web based interactive software for predicting the probability of occurrence of **15 nationally important livestock disease two months in advance**.
- The forecast can be viewed at www.nadres.res.in.

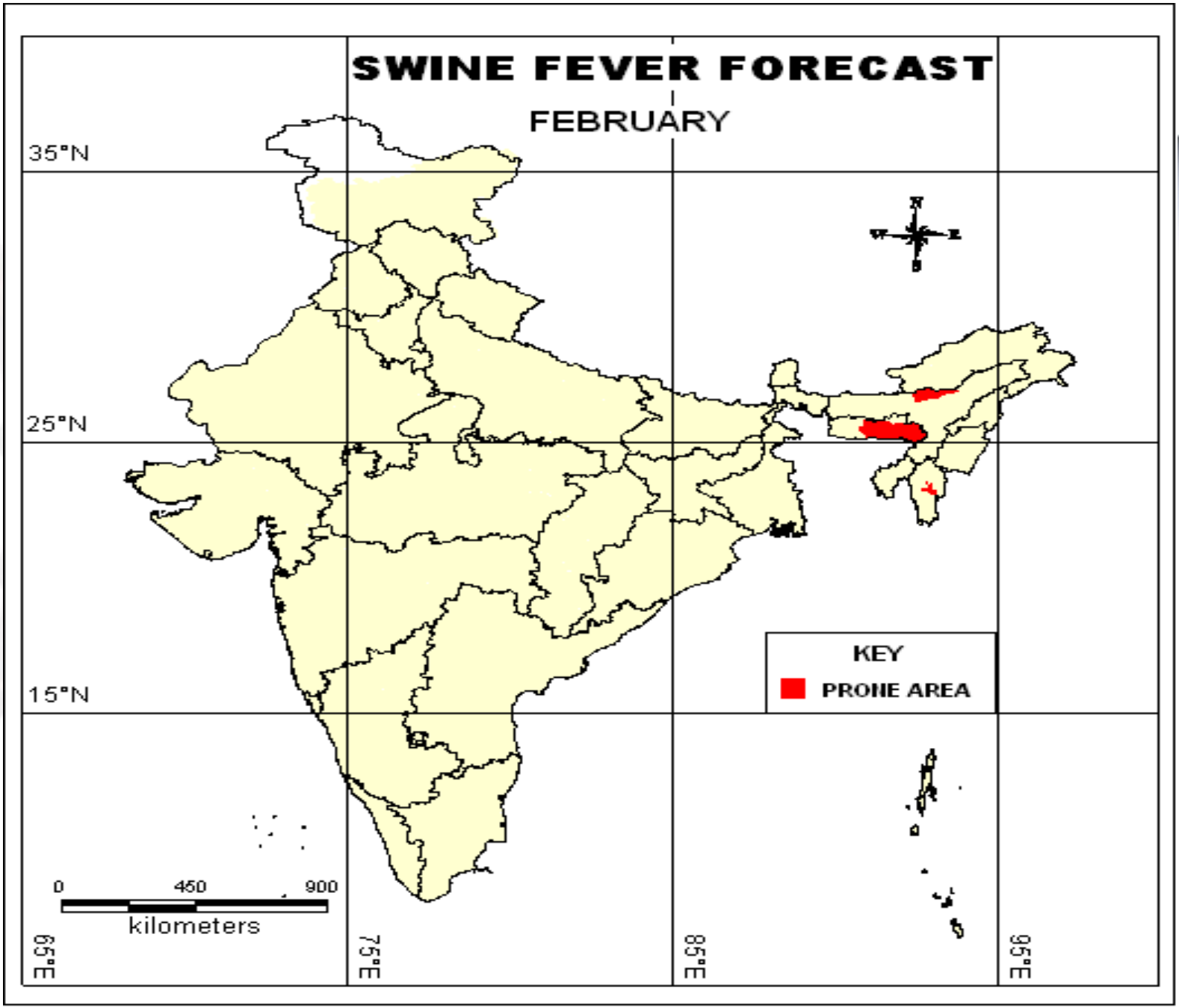






JANUARY FORECAST FOR ANTHRAX IN NORTH EAST ZONE OF INDIA





To improve animal disease surveillance systems Time to Time Education and Training are essential.

Overall, there is a need to shift from

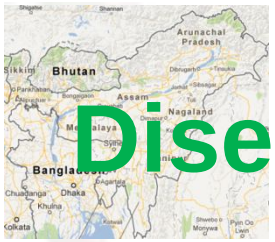
A Veterinarian
With a syringe

to

A Veterinarian
With a Strategy

Sampling strategies for infectious diseases

Dr. Arnab Sen
Head
Division of Animal Health



Disease surveillance

- **An effective animal disease control requires- an excellent knowledge of disease occurrence and distribution in time and space**

- **For a disease that is highly contagious or results in high mortality a permanent surveillance system is clearly required, so as to be able to detect the first outbreak whenever it occurs and then to monitor the situation from day to day**

- **Epidemiological surveillance is the method which best meets this need for information on a permanent basis**

- **Numerous countries, including some developing countries, have animal production and health information systems. These vary in complexity depending on the characteristics and performance of the country's livestock production system**

- **Each country must therefore determine its animal health priorities (eradication of a zoonosis, gaining external markets, etc.)**

- **On an international level, there has been growing interest in epidemiological surveillance since the signing of the World Trade Organization Agreement on the Application of Sanitary and Phytosanitary Measures**

- **The Office International des Epizooties (OIE) has been operating an international animal disease reporting system, comprising an alarm system for significant epidemiological events and the collection and dissemination of monthly and annual data on the occurrence of a limited number of diseases (known as List A and B diseases)**

- **Under this information system, the data to be submitted by a country are simple, or even minimal. Their main function is to give an overview of the general animal health status of the reporting country**

Defining the importance of diseases

- **When does a disease become important enough to warrant official intervention? Or to merit international attention? Much attention has been given to highlighting this issue in recent years.**
- **The International Office for Epizootics (OIE) has classified animal diseases into two "lists" - List A and List B in order to characterize their level of significance in terms of international trade.**

- The most important diseases are classified under LIST A. The definition of List A diseases is:
- **“Transmissible diseases which have the potential for very serious and rapid spread, irrespective of national borders, which are of serious socio-economic or public health consequence and which are of major importance in the international trade of animals and animal products”.**
- **List A diseases are**: Foot and mouth disease ,Vesicular stomatitis ,Swine vesicular disease, Rinderpest, Peste des petits ruminants, Contagious bovine pleuropneumonia, Lumpy skin disease, Rift Valley fever ,Bluetongue, Sheep pox and goat pox, African horse sickness African swine fever ,Classical swine fever, Highly pathogenic avian influenza ,Newcastle disease.

LIST B diseases

- Of lesser importance are the **LIST B diseases**. Their definition runs as follows: **"Transmissible diseases which are considered to be of socio-economic and/or public health importance within countries and which are significant in the international trade of animals and animal products."**

- This group includes such diseases as:
  Rabies, Heartwater, Tuberculosis, New and Old World Screw worm, Brucellosis, and many others.

- **Events such as the BSE epidemic in Europe and the outbreaks of Nipah virus in Malaysia shown that even "unclassified" diseases can have severe economic or trading implications, especially when there is a link to public health.**
- **FAO/EMPRES: a new emphasis: On taking office in January 1994, the Director-General of FAO decided to focus in championing the goal of enhanced world food security and the fight against transboundary animal diseases and plant pests as outbreaks of such diseases or pests can result in food shortages, destabilize markets and trigger trade measures.**

- A new programme with two sub-components was established: i) to combat plant pests and diseases,

  ii) to fight livestock diseases

- These programmes fell under the umbrella of EMPRES - Emergency Prevention Systems for transboundary diseases of animals and diseases and pests of plants.
- This put livestock diseases something of a different light: transboundary diseases were now a specific target, and they are defined thus:

Transboundary diseases

"Those diseases that are of significant economic, trade and/or food security importance for a considerable number of countries; which can easily spread to other countries and reach epidemic proportions; and where control/management, including exclusion, requires co-operation between several countries"

- EMPRES has classified transboundary animal diseases into three flexible categories

Transboundary diseases three flexible categories

- **Epidemic diseases of strategic importance**, namely rinderpest, foot-and-mouth disease and contagious bovine pleuropneumonia (CBPP) - these are accorded top priority by EMPRES at the global level. However, regions or countries can have a country-/region-specific set of strategic diseases, as well.
- **Diseases requiring tactical attention at the international/regional level**, **e.g.** Rift valley fever, lumpy skin disease, Peste des Petits Ruminants (PPR), Newcastle disease, African swine fever (ASF) and classical swine fever.
- **Emerging or evolving diseases, e.g.** BSE, porcine reproductive and respiratory syndrome (PRRS)

Primary role of surveillance

- **Where a disease is unknown in an area or has been absent for a long time, only one or two cases may qualify as an epidemic and warrant immediate attention.**
- **Where a disease has been present at a fairly constant prevalence level for some time, a marked upswing in the number of cases seen may signal a change in status from endemic to epidemic and will require investigation.**
- **Thus the primary role of surveillance is to detect these changes in status early enough to take action.**

- **It means having the ability to detect a new incursion, or changes in present status, and presents a challenge to veterinary services in countries around the world.**

- **Renewed attention is being given to Transboundary Animal Diseases (TADs), many of which have their greatest impact in those very countries where surveillance (for many reasons) may be weakest**

- **The key to success in handling animal disease epidemics is early detection**

Early Detection

- If a disease can be detected very early in the phase of epidemic development, the possibility exists that it can be arrested and eliminated before it actually inflicts damage
- Early detection presupposes that there is a surveillance system in place that will bring infection to light when it is first seen.
- Early detection enables early warning and an early reaction. Surveillance is the primary key to effective disease management
- Surveillance plays an important role in the monitoring of progress in control and eradication programmes

What is surveillance?

- The word "surveillance" has been used by epidemiologists for some considerable length of time, often interchangeably with "monitoring", and it is only recently that serious thought has been given to defining the two words
- Surveillance may be thought of as having a broad definition, in the sense of watching a population closely in order to see if a disease makes an incursion. The object of surveillance is early detection of disease.
- **"All regular activities aimed at ascertaining the health status of a given population with the aim of early detection and control of animal diseases of importance to national economies, food security and trade".**

Monitoring

- **Monitoring, on the other hand is a more specific activity/ies that will follow as part of an early reaction should surveillance activities indicate introduction of disease**

- **It will focus more specifically on the identified disease in order to ascertain changes in prevalence level, rate and direction of spread. Monitoring can thus be defined as:**

- **“All activities aimed at detecting changes in the epidemiological parameters of a specified disease”**

- **For sero-surveillance inputs (aggregation of these data at village/district level is satisfactory for a national database; information about each individual**
- **Data items for each observed outbreak would be sufficient:**

- **Locality* Date***
- **Georeferences* Species***
- **No. of cases* No. of deaths***
- **No. of animals at risk**
- **No. of animals examined**
- **History and/or clinical signs Clinical examination/field tests**
- **Disease history of herd/flock**
- **Vaccination history (past yr) Age/sex category/ies bled**
- **Tentative diagnosis* Age category most affected, Sex category most affected**
- **Any treatment given**
- **Any post-mortem lesions seen, any samples to a laboratory**
- **Laboratory Results if available, Farming system**
- **Name of reporting officer**

- **Data requirements from abattoirs or slaughter slabs should include the following:**
- **No. of animals in consignment (where applicable)**
- **Origin of animal/s (where known)**
- **Lesions seen Condition diagnosed**
- **Age most affected, Sex most affected**
- **Samples sent to lab Laboratory results if available**
- **Category of reporting officer**

Surveillance and monitoring systems for animal health programs

- Information about the health-related event might be collected from owners by interview or mail.
- Biological samples might be collected during farm visits or at abattoirs, knackeries, or carcass rendering plants.
- In addition, the screening of animal medical records (either the files or electronic databases) for specific entries or the screening of biological sample banks for specific pathogens or lesions can be considered part of the active collection of data

Surveillance and monitoring systems in India

- **National Animal Disease Reporting System (NADRS), by networking about 7100 offices of the Animal Husbandry and/or Veterinary offices located at Block, District, State and Central level involved in for monitoring and surveillance about 143 animal diseases and their control, in the country (under DADF)**
- **Animal Disease Diagnostic Laboratories Workflow Application (ADDLWFA) has been designed and developed under the NADRS Project to integrate the results of tests conducted on samples by the Animal Disease Diagnostic Laboratories (ADDLs), which are about 970 in numbers, available in the country.**

- **The project on animal disease monitoring and surveillance, which was initiated by the ICAR in the 7th five year plan as an All India Coordinated Research Project (AICRP) became fully functional during the last quarter of 1987 with the establishment of four Regional Research Units (RRUs), located at Bengaluru, Hyderabad, Pune, and Ludhiana.**
- **Realizing the impact of animal disease monitoring and surveillance on livestock sector, ICAR upgraded this project to an independent institute status as Project Directorate. At present, the Directorate is working with fifteen collaborating units on animal disease monitoring and surveillance.**

GENERAL CONSIDERATIONS FOR COLLECTION OF SPECIMENS

- Collection of clinical samples is done primarily for four reasons- 1.Direct examination (rapid diagnosis), 2.Isolation of causative microorganisms, 3. Serological investigation and 4.Diagnosis of the disease

- **Collection of samples:**

1. Collect blood samples from ailing, few in-contact and recovered animals.
2. In case of deaths, necropsy should be performed and samples should be collected aseptically for isolation of the causative agent and histopathological (H.P) examination.
3. Samples should be collected before the treatment is initiated.
4. All containers and instruments should be sterilized before collecting samples.

5. **Skin surface should be cleaned with the antiseptic solution for the collection of samples like skin scrappings, pus material, etc.,**
6. **Necropsy samples should be collected from the centre of the organs after singeing the surface with a hot spatula.**
7. **Label all the specimen containers :**

   Species of animal…….., **Date of collection……..,**

   Preservative added if any……., **Type of specimen……..,**
8. **A brief history of the disease outbreak should accompany the sample as follows;**

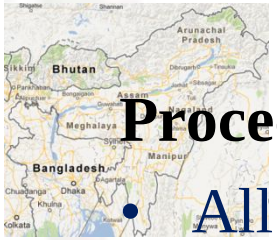
   Town/village……., **Species of animal…….,**

   Other species infected if any……,

   Population size…….., **Animal no……..,**

   No. animals affected……., **Sporadic or epidemic…….,**

   Vaccination/Treatment, if any…….. **Other information, if any**



Procedure to be followed for dispatch of clinical samples

- All sample containers should be labeled as **“Biological Specimen” “ Fragile ,handle with Care”.**
- Biosafety measures (protective clothing, mask, gloves and laminar workstation) should be followed while collecting and dispatching specimens from animals suspected for rabies , anthrax and other zoonotic diseases
- Whole blood may be despatched by mixing with an appropriate anti-coagulant (EDTA, sodium citrate, etc.).
- Serum samples should be despatched without adding any preservative.
- Blood smears should be despatched after fixing with 70% alcohol.
- The tissue specimens and swabs for isolation of virus should be collected in 50% glycerine phosphate buffered saline or phosphate buffer solution containing 5% bovine serum albumin and antibiotic (e.g., gentamicin @ 50 µg/ml) or commercial viral transport media.

- For Isolation of bacteria, specimens should be despatched in transport medium to reach laboratory on ice at the earliest.
- Organ samples should always be despatched in **transport media or directly on ice** as quickly as possible to prevent autolysis.
- Urine samples for bacteriological study should be collected in sterile containers **without any preservative and despatched within 1 hour**
- Faecal samples should be despatched in screw cap metal or plastic containers preferably on ice.
- Milk samples for bacteriological study should be despatched as quickly as possible. If the despatched is delayed, then they should be despatched by adding preservatives such as boric acid at the rate of 1 part of 50% boric acid to 10 parts of milk.
- Cerebrospinal fluid, synovial fluid and fluids collected from the thoracic, abdominal or pericardial cavities should be submitted in sterile screw cap **without adding any preservative.**

Early detection of a disease presupposes that there is a surveillance system in place

Thank you

MICROBIOLOGY LABORATORY

Dr N. N. Barman
Dr Pankaj Deka
Dr Sophia M Gogoi
Dept of Microbiology, C. V. Sc, Khanapara

Microbial Materials

BioHazardous Agents

Pathogens that can replicate & cause disease

- Bacteria (*Streptococcus pyogenes)*
- Fungi (*Candida, Histoplasma)*
- Viruses (HIV, HBV)
- Prions(CJD)
- Parasites (*Giardia, Strongyloides)*

Toxins – Microbial poisons

- Exotoxins – produced by bacteria
  - *Clostridium botulinum - food poisoning, one of most deadly*
  - *Clostridium tetanii – tetanus*
  - *Corynebacterium diphtheriae – diptheria*

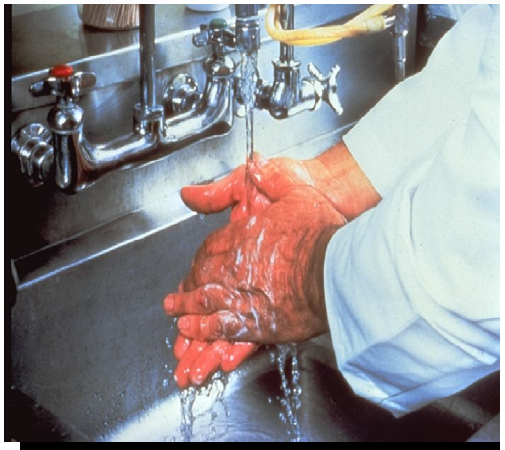
Bioterrorism use

- Endotoxins – released from cell wall when bacteria disintegrates

Biosafety Concepts

1. Standard Microbiological Practices

- Access to laboratory limited to trained personnel
- Lab coats, gloves and Eye protection worn at all times
- Workers should wash their hands after any work
- Eating, drinking , smoking, applying cosmetics in lab prohibited



Biosafety Concepts

1. Standard Microbiological Practices

- Hand to mouth or hand to eye contact avoided
- Mouth-pipetting prohibited
- Minimize aerosol production

Biosafety Concepts

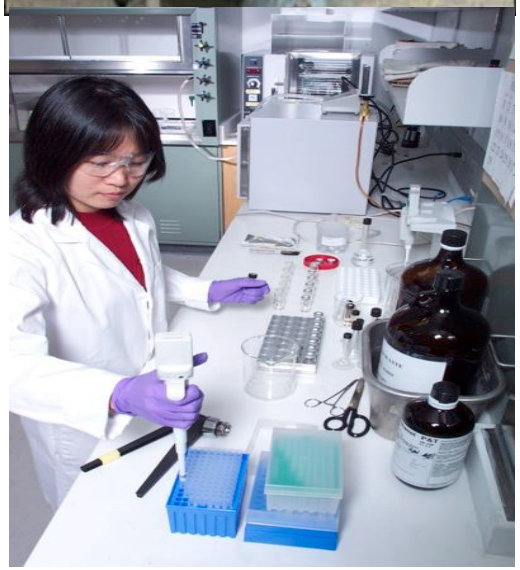
1. Standard Microbiological Practices

- Work performed on clean impervious bench surface , appropriate disinfectant at hand
- Work surfaces decontaminated after any spill, at end of every work session
- All biological materials properly decontaminated before disposal

(2) Safety Equipment

Primary Containment Barrier

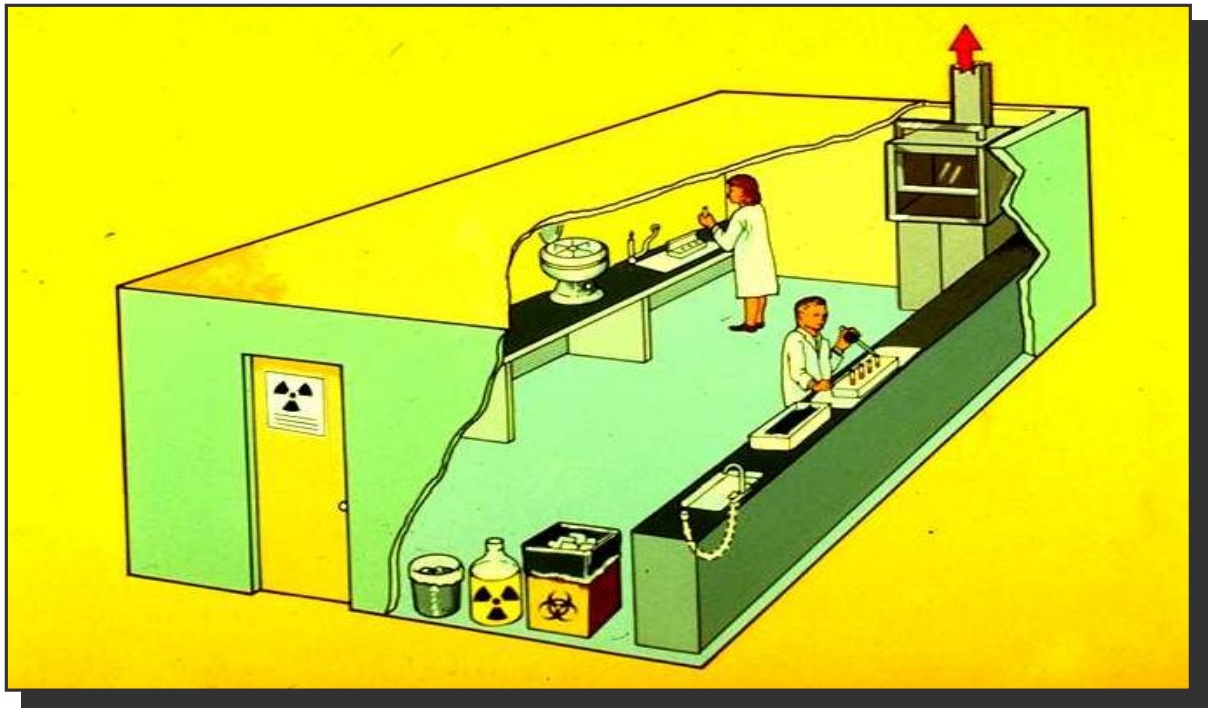
- protection of personnel from exposure to infectious agents
  - Gloves, gowns, Respirator
  - Face shield, Booties



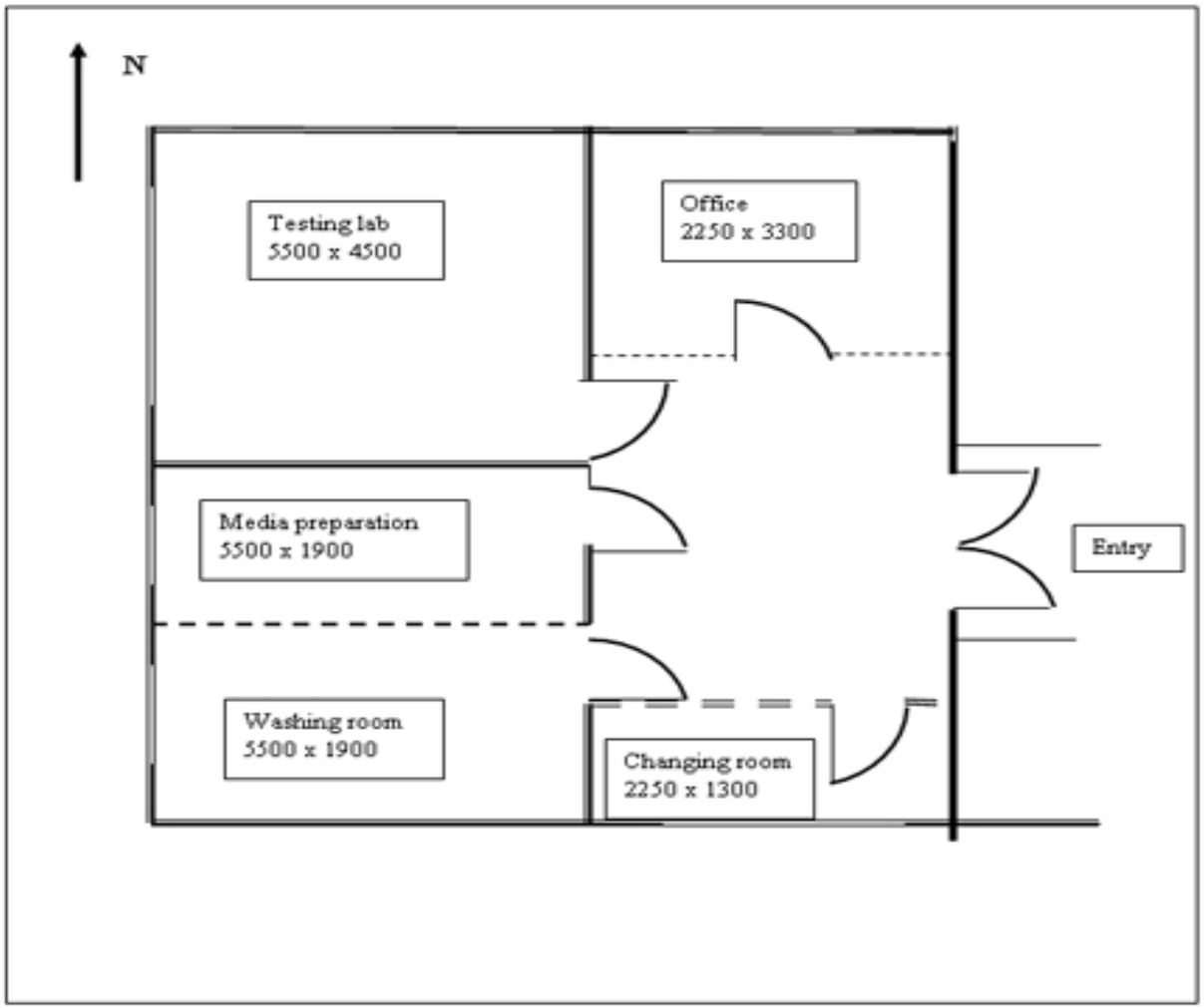
(3) Facility Design and Construction

- Contributes to worker protection
- Protects outside laboratory (Environment & Neighborhood)
  - Building location & Lab design
  - Ventilation
  - Autoclaves
  - Cage wash facilities

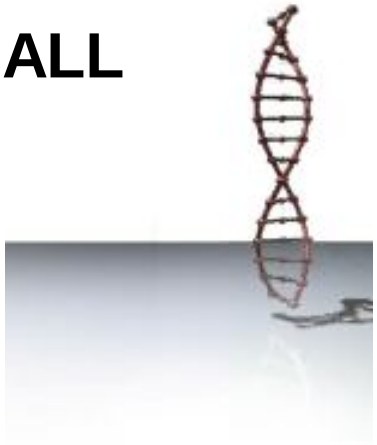
Laboratory layout

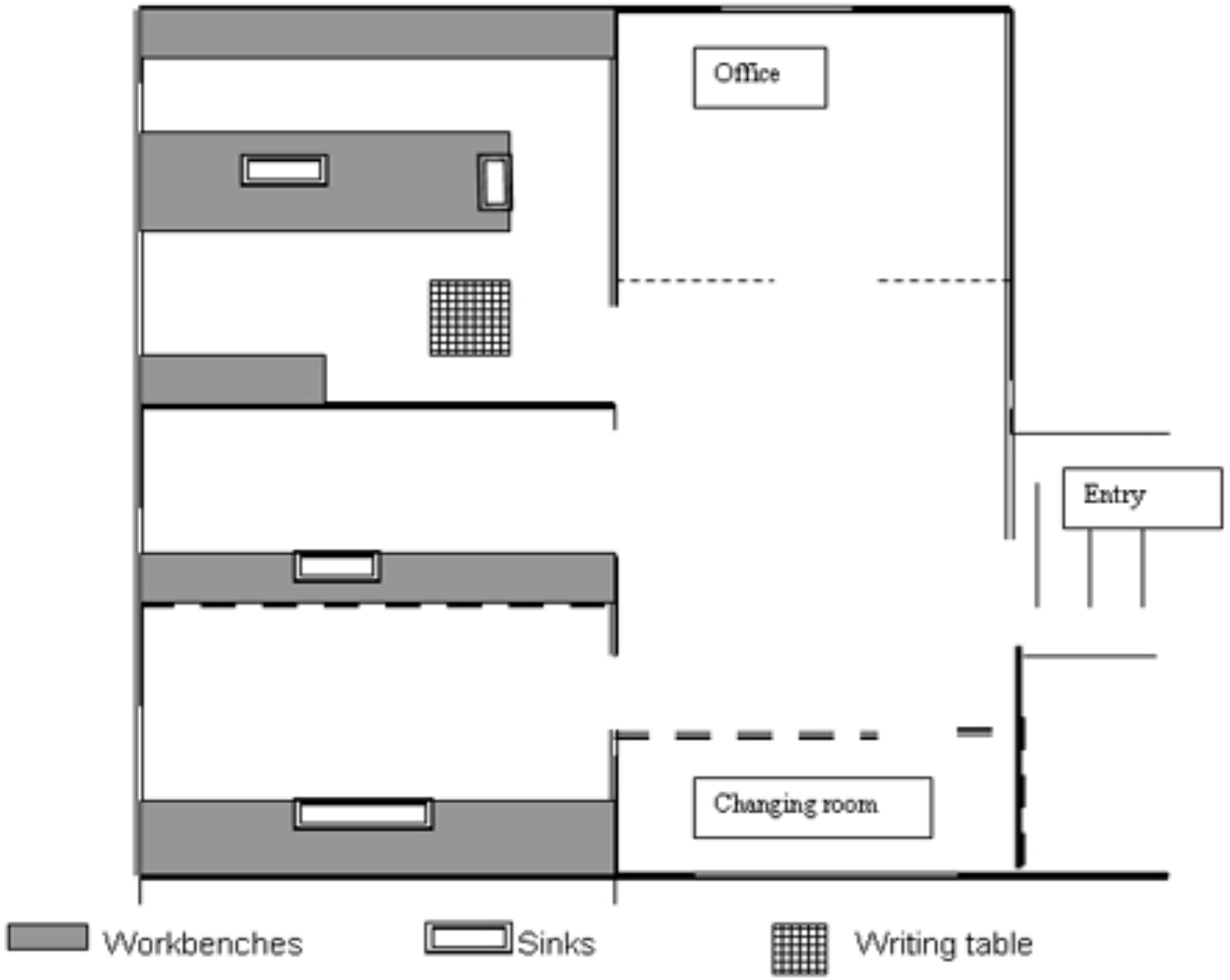


LAYOUT PLAN FOR A SMALL MICROBIOLOGICAL TESTING LABORATORY

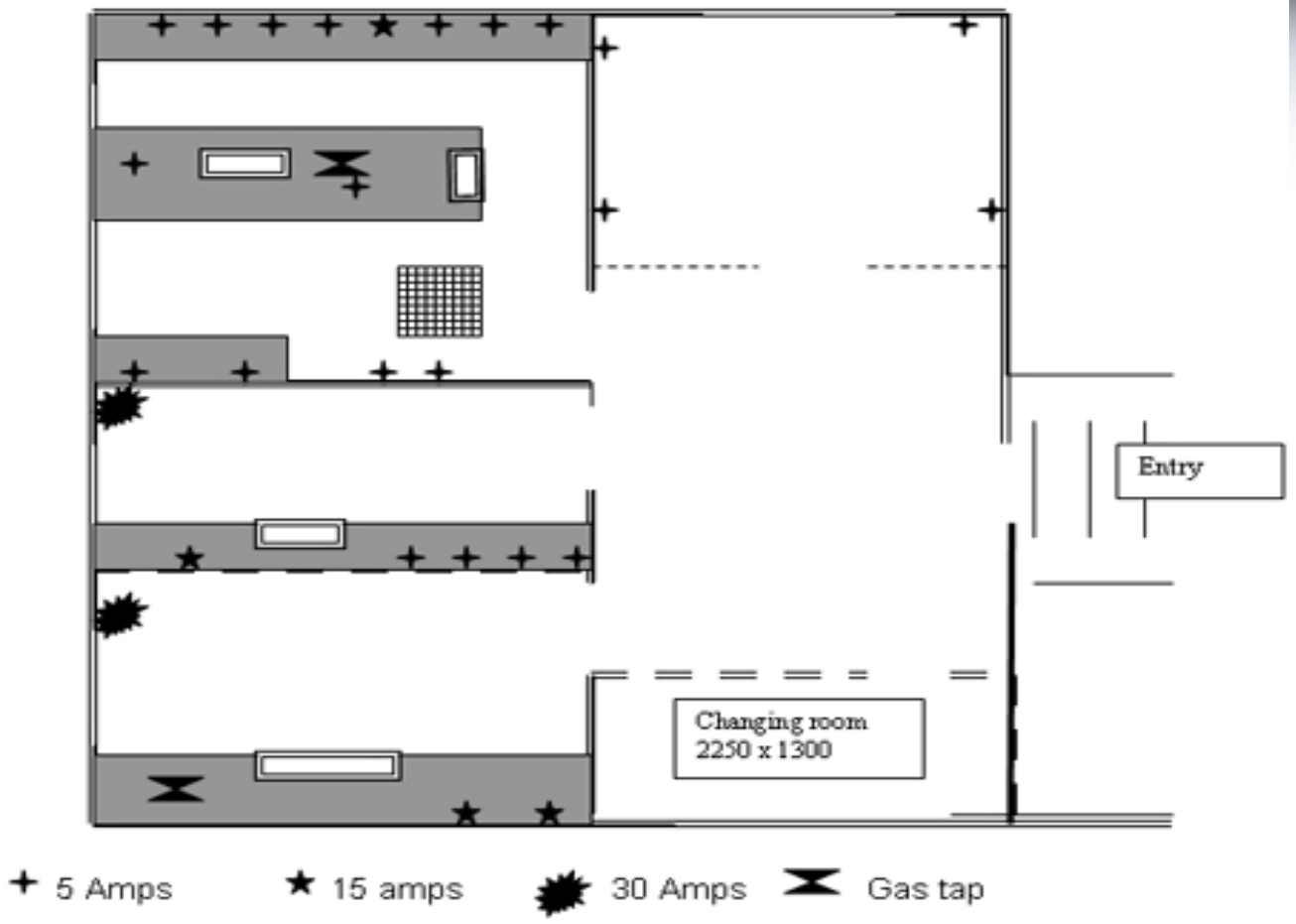


LAYOUT PLAN FOR WORK BENCHES IN A SMALL MICROBIOLOGICAL TESTING LABORATORY

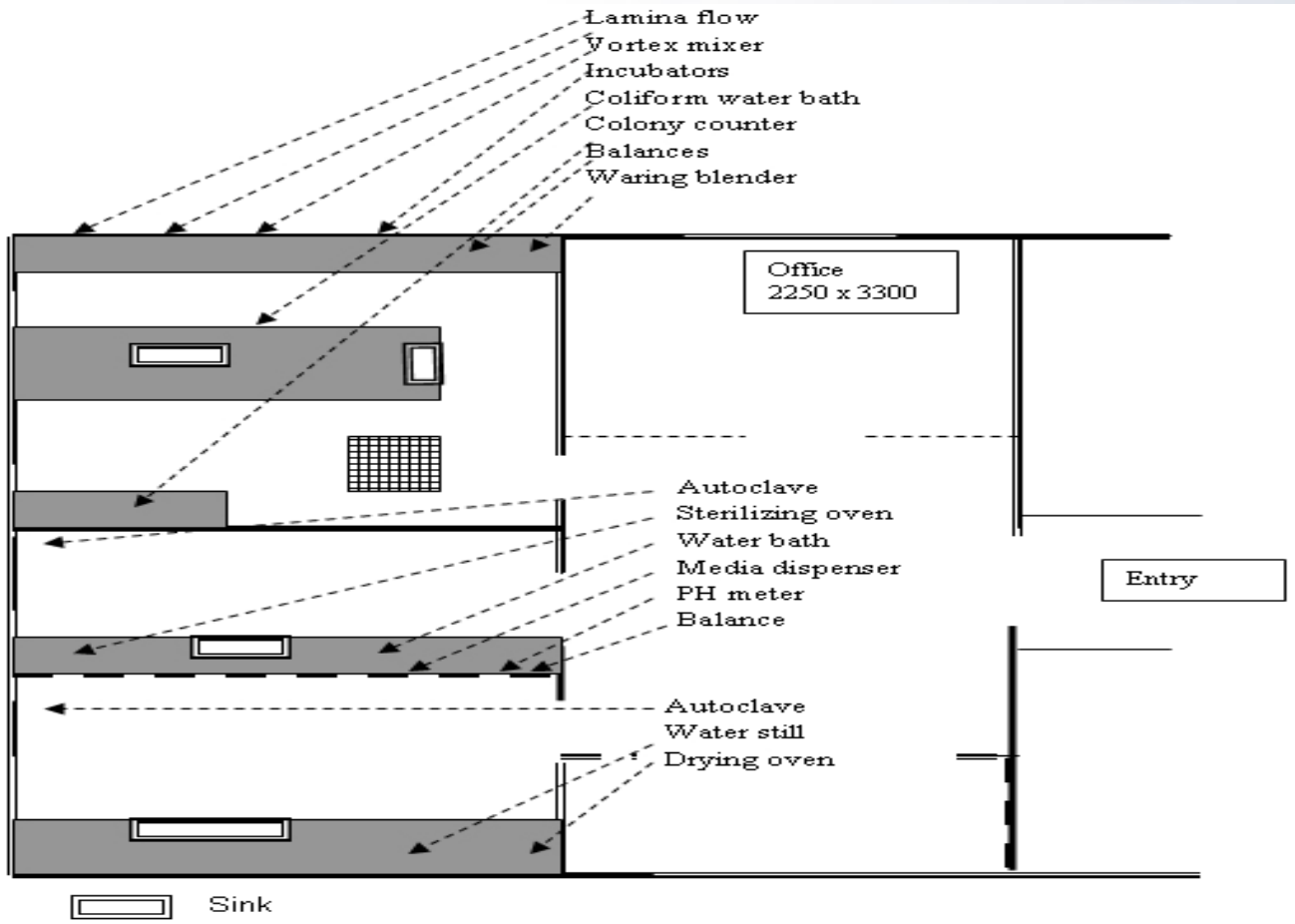




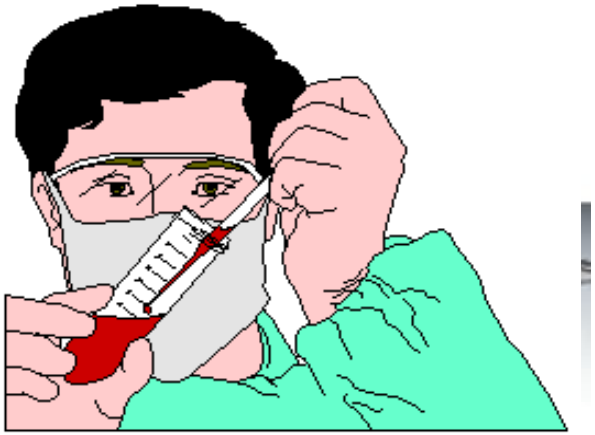
LAYOUT PLAN FOR ELECTRICITY AND GAS SUPPLIES IN A SMALL MICROBIOLOGICAL TESTING LABORATORY



LAYOUT PLAN FOR EQUIPMENT FOR A SMALL MICROBIOLOGICAL TESTING LABORATORY

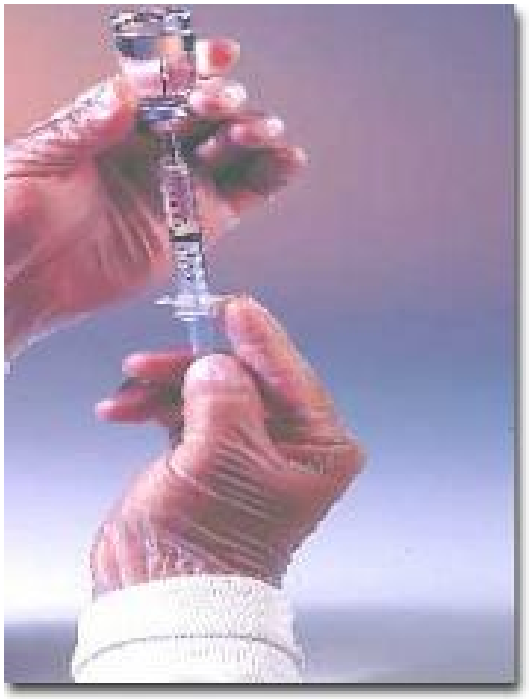


Practices & Procedur



Special Practices

- Agents associated with human disease
- Treatment for disease available
- Agent poses moderate hazard to personnel and environment
- Direct contact or exposure
- Percutaneous exposure
  - Scratch, Puncture, Needle stick
- Mucus membrane exposure
  - Eyes, Mouth, open cut



Special Practices

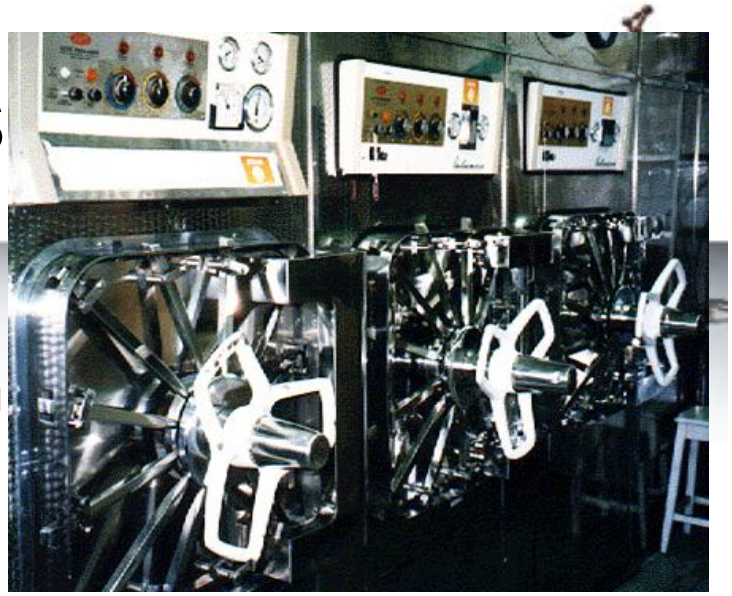
- Needles & Sharps Precautions
  - Use sharps containers
  - DON'T break, bend, re-sheath or reuse syringes or needles
- DON'T place needles or sharps in office waste containers
- DON'T **t**ouch broken glass with hands

Biological Waste

Types

- Cultures, stocks, isolates
- Materials contaminated with blood
- Sharps
- Pipettes, wrappers, tips
- All material used in lab
- Disposal-Contact EHS for pickup
- Disposal animal waste
  - Puncture-proof, leak-proof, sealable receptacles
  - Avoid overfilling
  - No biologicals can go down drain

Decontamination Methods



3 Types

- Heat
  - Steam sterilization
  - Dry Heat sterilization
  - Incineration

- Chemical
  - Liquids, i.e. phenol, sodium hydroxide, hydrogen peroxide
  - Gases, i.e. ethylene oxide

- Radiation

Lab Decontamination

- General Lab Use – Hypochloride Solutions (Bleach)
  - Large Spills/Large Organic Load-undiluted
  - Small Spills/Virus Inactivation – 10%- 1:9/2-4% NaOH
  - General surface Disinfection – 1%- 1:99/ Phenol

A list of disinfectants which are active against *Avian Influenza* virus, their concentration and recommended use

- Rectified spirit or Savlon or Dettol (1% solution) can be used for cleaning of hands, feet of farm workers and visiting officials.
- 2% solution of NaOH should be used at the entrance on foot mats to clean the shoes. This solution can also be used to scrub and clean gumboots and other items.
- Sodium hypochlorite 2% solution: active chlorine solution (disinfection of equipment)
- Quaternary ammonium salts 4% solution: (treatment of walls, floors, ceilings and equipment).
- Calcium Hydroxide 3% solution: (treatment of walls and floors).
- Cresolic acid 2.2% solution: (treatment of floors).
- Synthetic phenols 2% solution: (treatment of floors).
- Vircon-S @ where available.
- Formalin and potassium permanganate for fumigation.

Autoclaving Biohazardous Waste

•Wear buttoned lab coat,
–eye protection
– closedtoed shoes
–heat resistant gloves

• Add 1 liter of water
–to properly vented clear or orange autoclave bag by
–pour carefully down the side of the bag

• Use
–a solid secondary container
–or pan approved for autoclave use

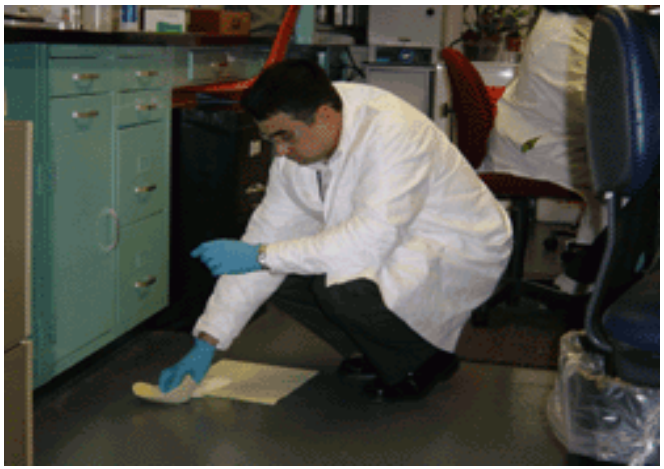
• Promptly remove cooled autoclaved biohazardous waste to trash

• Record use in user log

Accidental Spills

- Evacuate area, alert personnel and cordon off so that aerosols may settle
- Don PPE; Cover with paper towels and apply bleach (1 part bleach : 9 parts water
- Allow 15 – 20 min contact time
- Wipe up working towards center
- Use tongs/Forceps if broken glass is involved

CREATING A BIOLOGICAL SPILL KIT

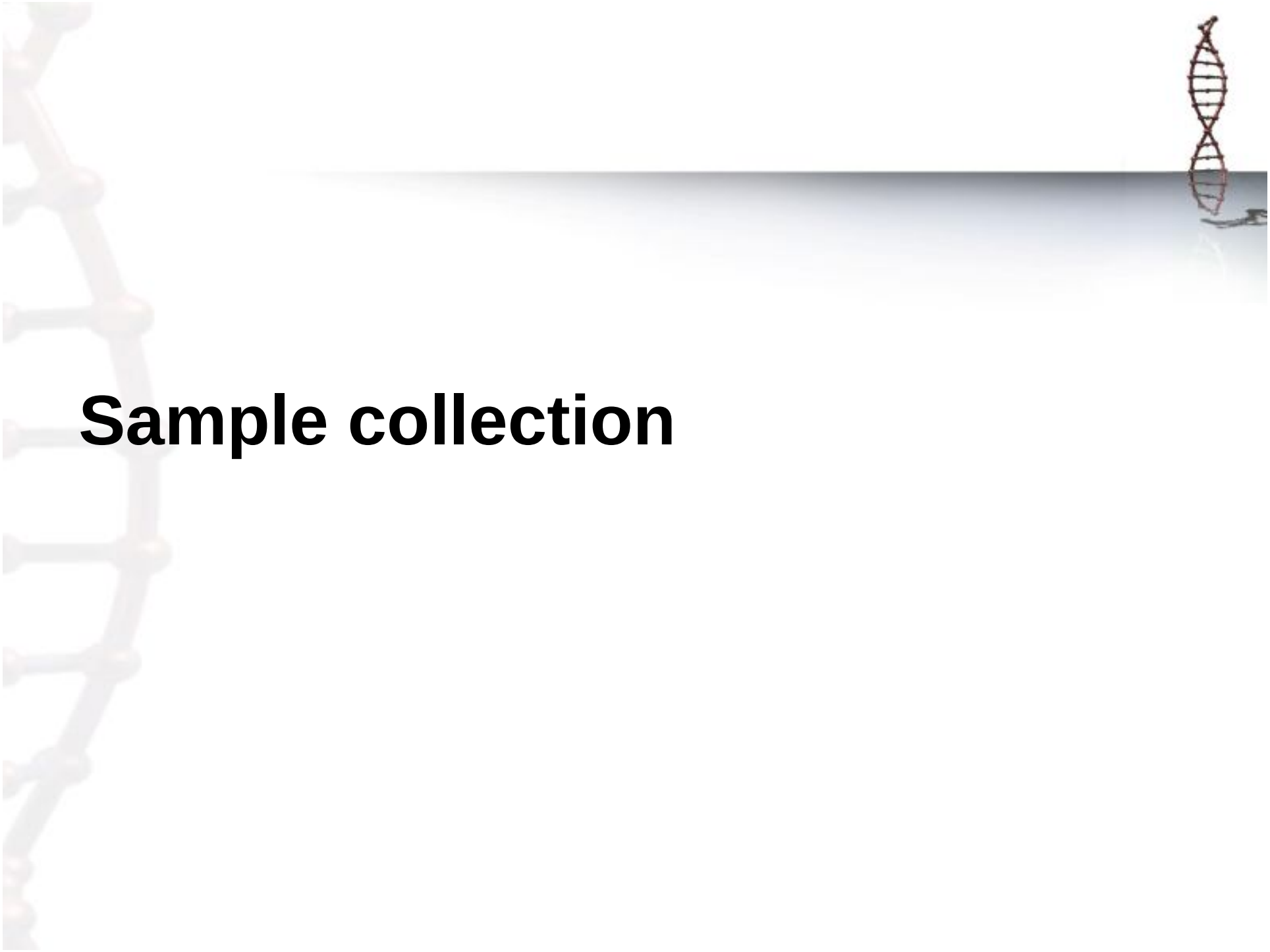


First Aid Measures

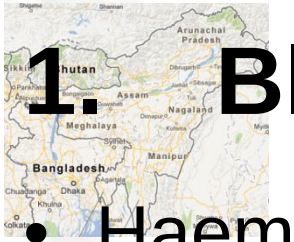
- Splash to Eye or Needle stick Injury
  - Rinse thoroughly for **15 minutes** at the eyewash or sink

Documentation and Maintenance of Laboratory Records

- To ensure the availability of the data needed for validation, review and statistical analysis
- To ensure that authorized persons have all the information necessary
- To define the specifications and procedures for all materials and methods



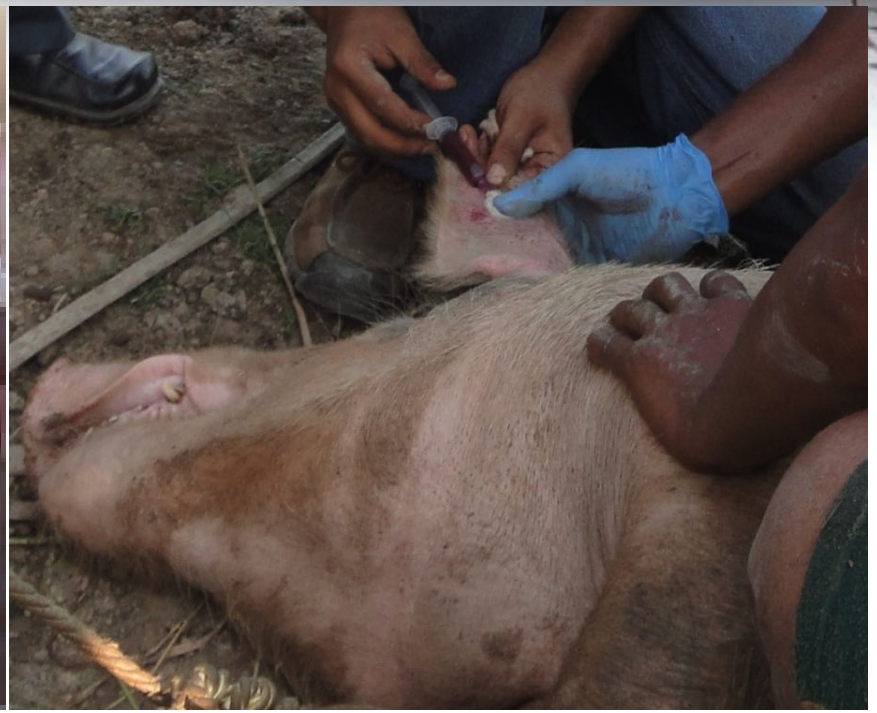
Sample collection



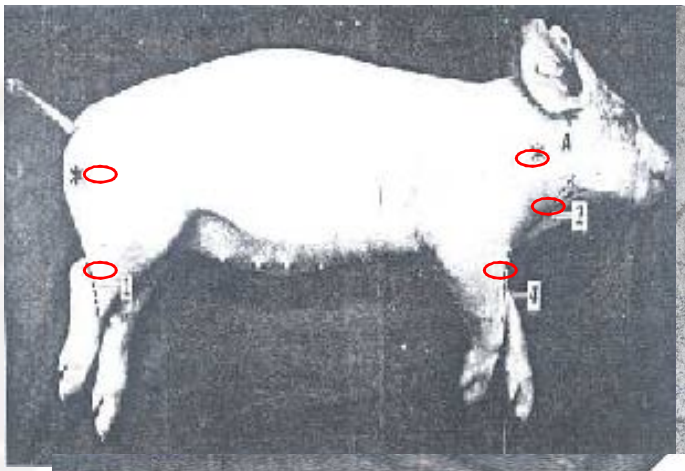
1. Blood

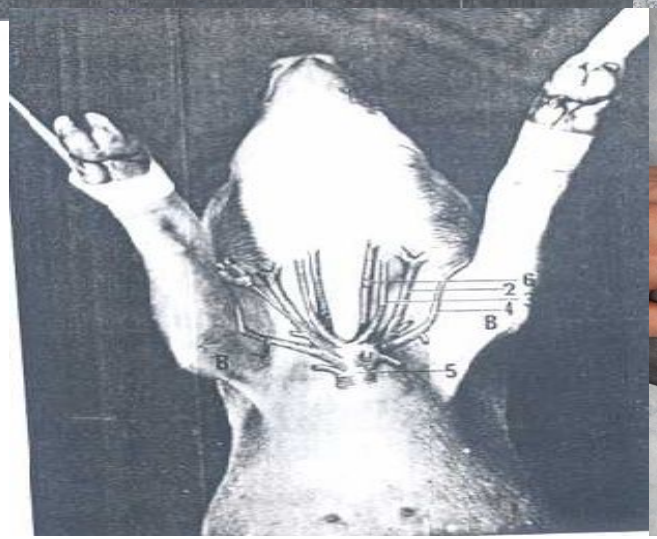
- Haematology or for culture and/or direct examination for bacteria, viruses, or protozoa, in which case it is usual to use anticoagulants

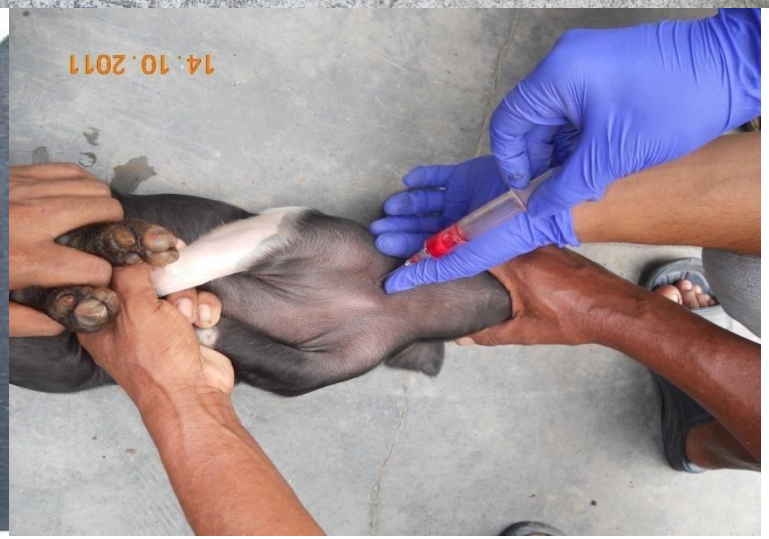
Requirements



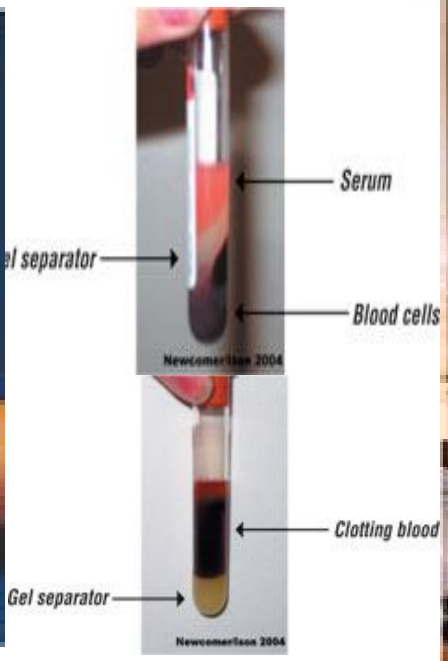
Collection of Blood & Injections

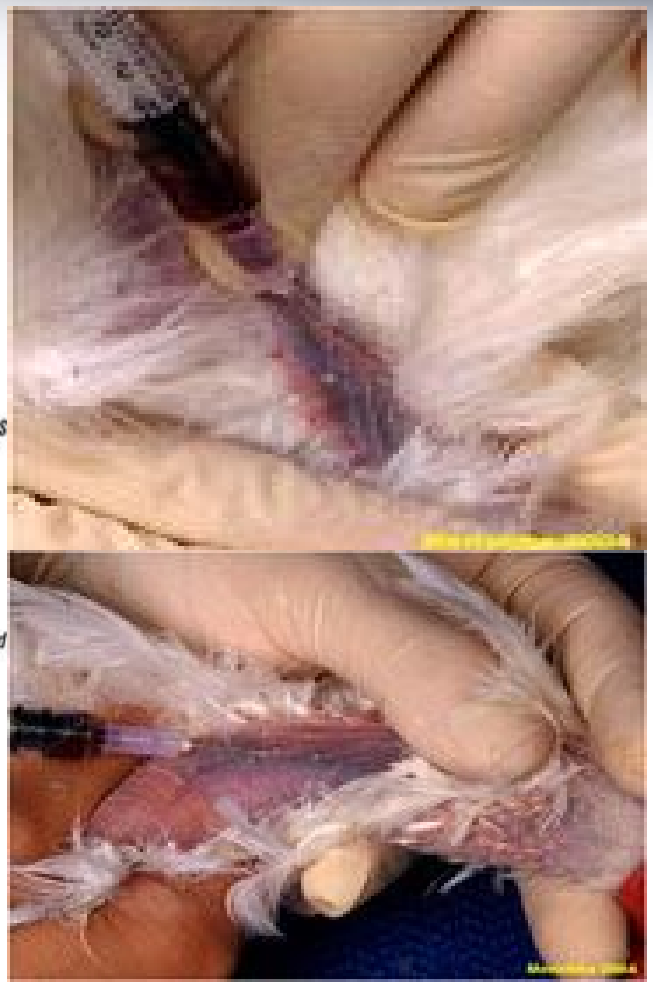




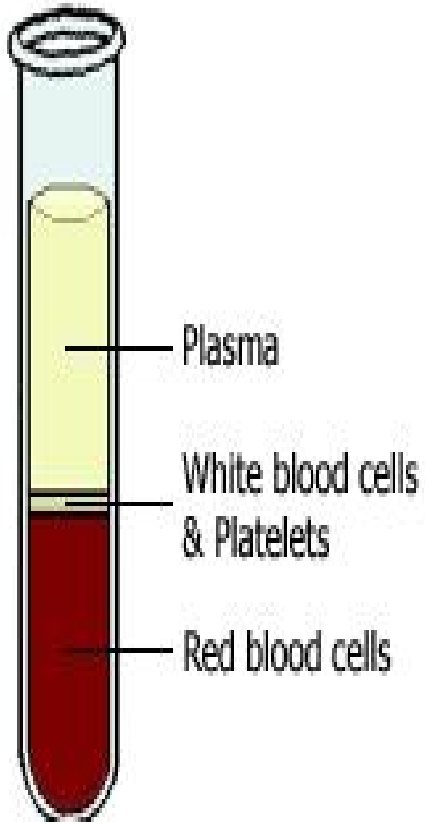


Collection of Blood from Birds



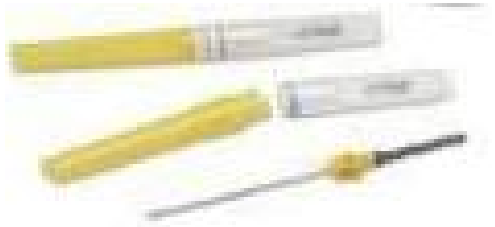


Blood after centrifugation

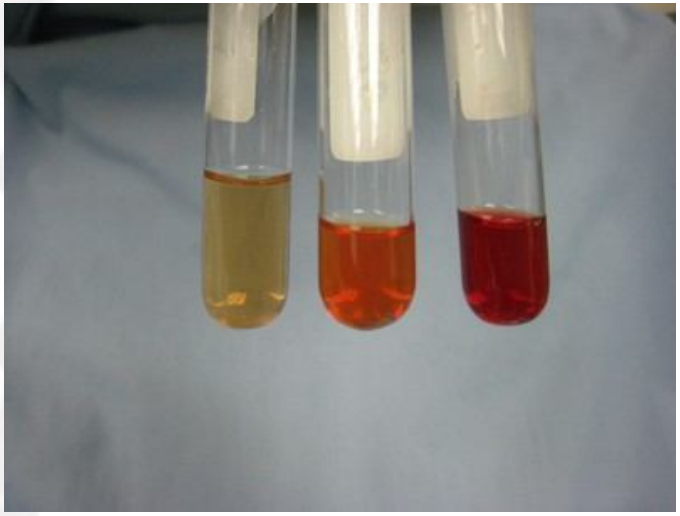


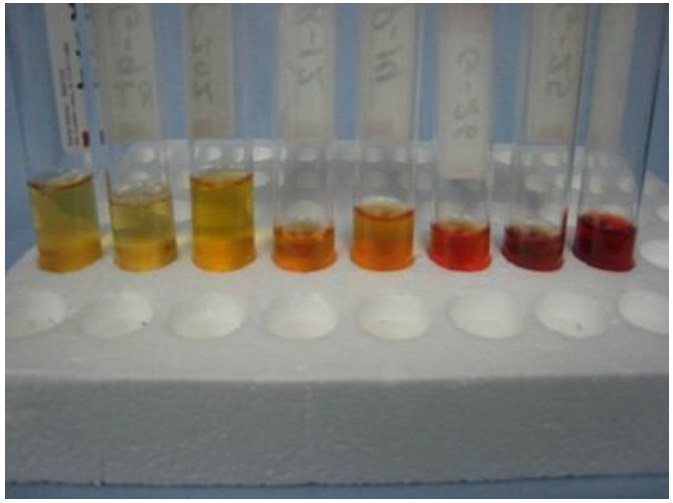
2. Serum

- Serology – clotted sample is necessary for extraction of the serum



VARIOUS STAGES OF HEMOLYSIS IN SERUM SAMPLES





3. Faecal sample

- Freshly voided faeces of suitable quantity (about 5-10 g) should be selected, and sent with or without a transport medium.

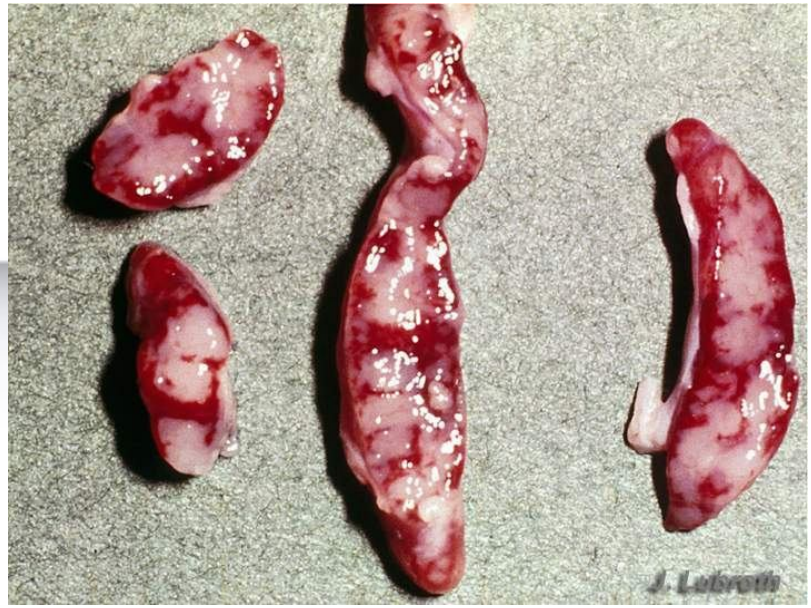
- Faeces for Parasitology should fill the container or be topped up with sterile water to reduce air and prevent hatching of parasite eggs.

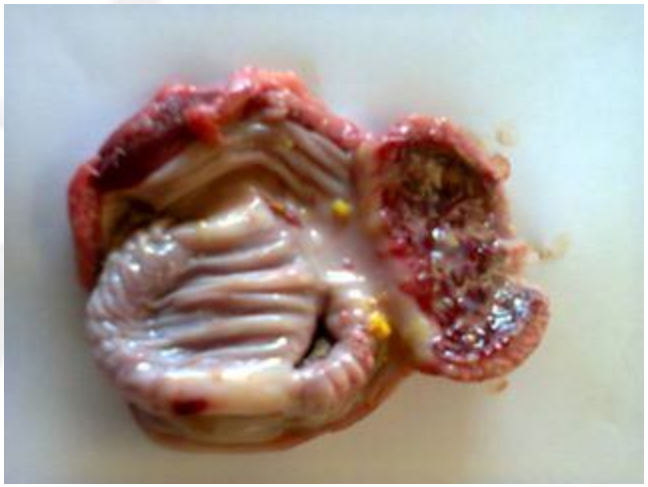
4. Skin

5. Discharges

6. Milk

7. Tissue Sample





Dispatch of samples

- Clear labeling
- Careful handling
- Coolant/Gel packs to maintain temperature

The following must strictly be avoided

Bacteriological Examination

Direct Microscopic Examination

Cultural Examination
Isolation

Antibiogram

Virological Examination

Isolation
&
Identification

Serology: AGPT
HA/HI, ELISA

Fungal Examination

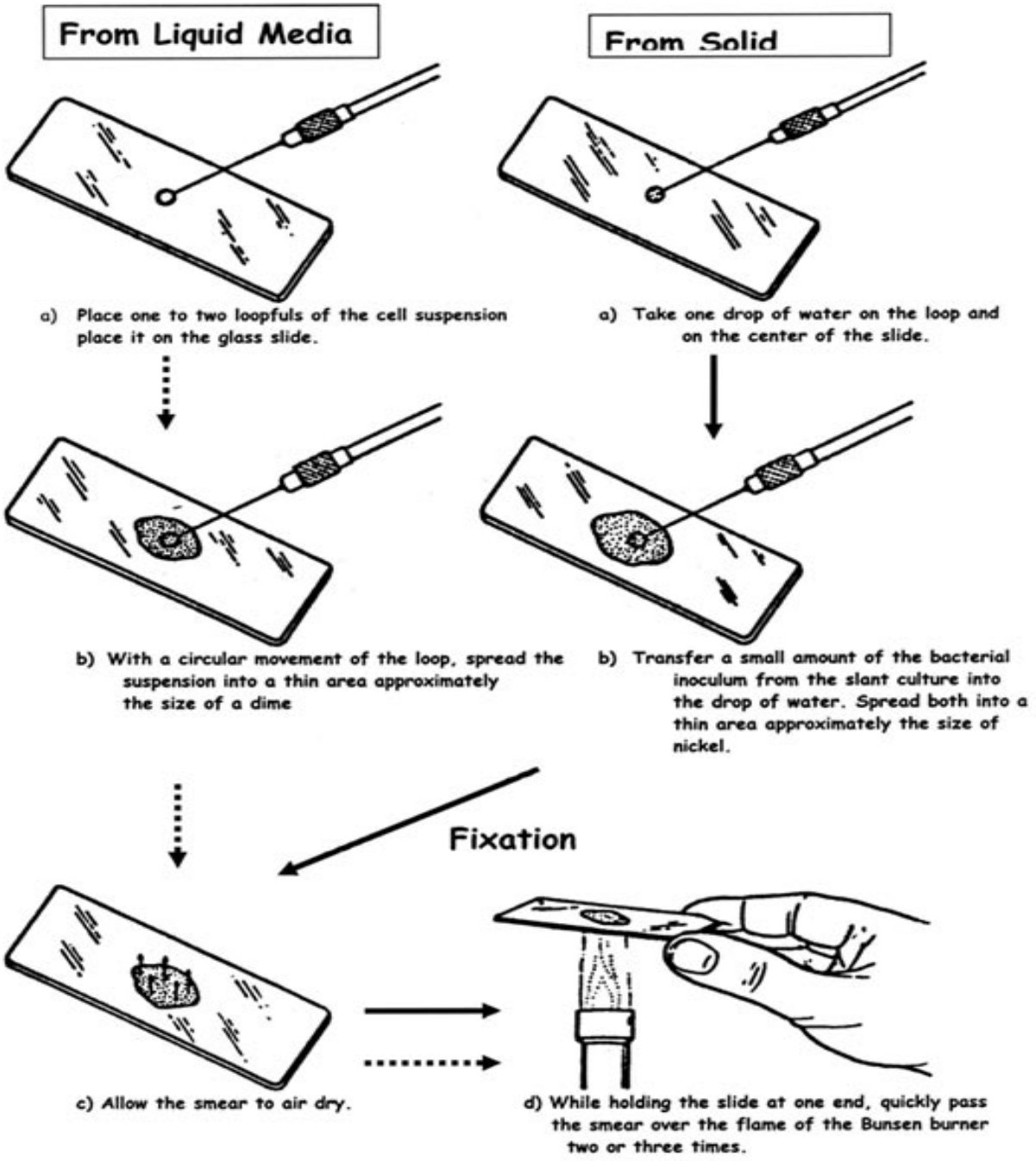
Direct Microscopic Examination

Cultural Examination
Isolation

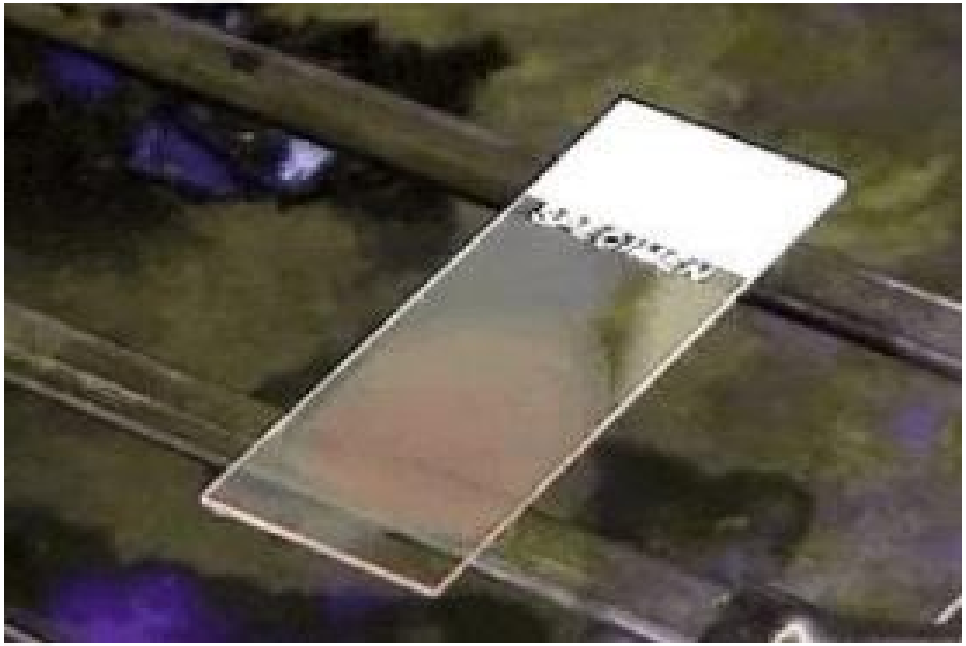
Requirements- For Staining

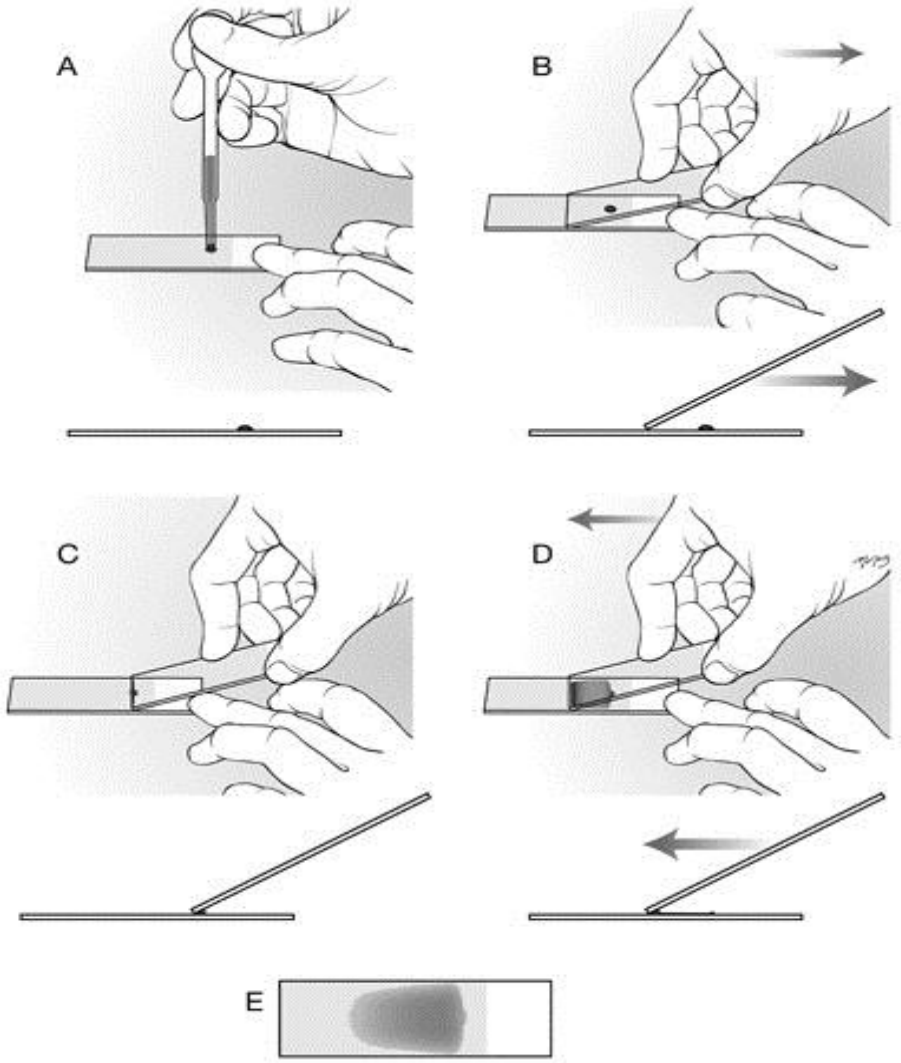
Sl. No	Materials	Source
1	Inoculating loop	Tarsons HIMedia Borosil SISCO Research Lab
2	Micro slide	
3	Spirit Lamp	
4	Staining Rack	
5	Microscope	
6	Staining Kit (Methylene Blue, Gram's Stain, Acid Fast Stain)	
7	Seeder wood oil, Xylene & Cloth (Ganji)	
8	Glass marking pencil	

Preparation of Smear from bacterial culture



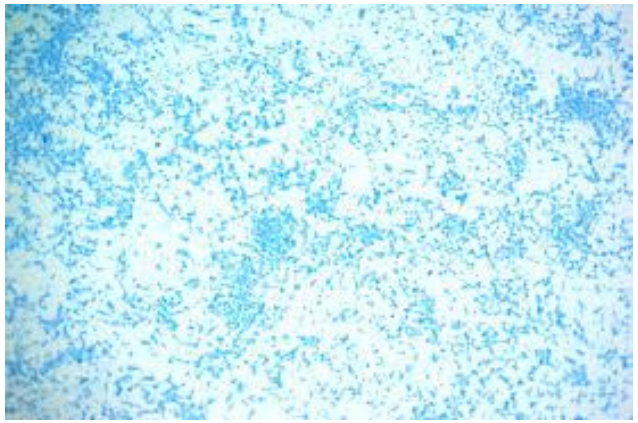
Preparation of Smear from blood



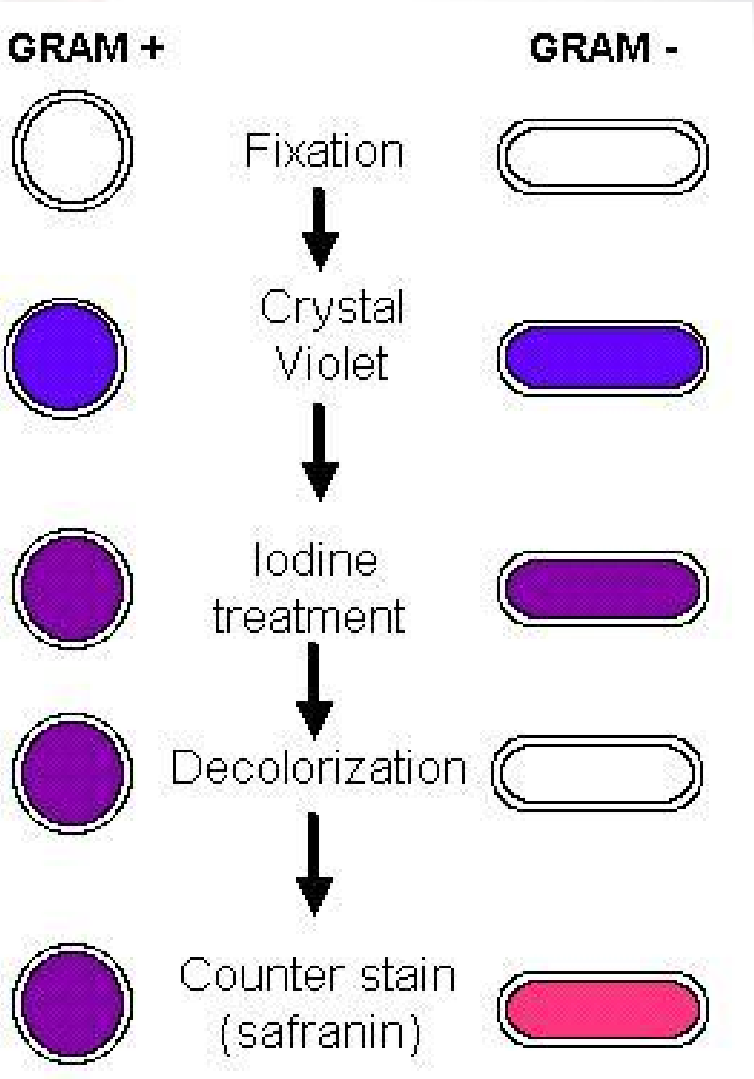


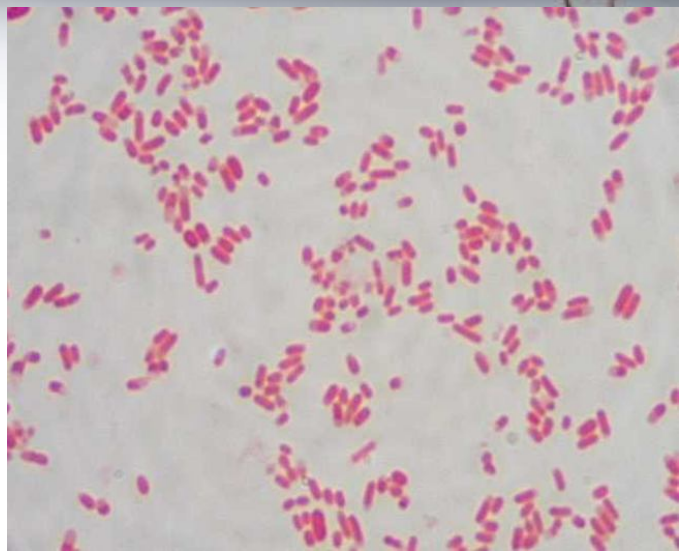
Methylene Blue Staining

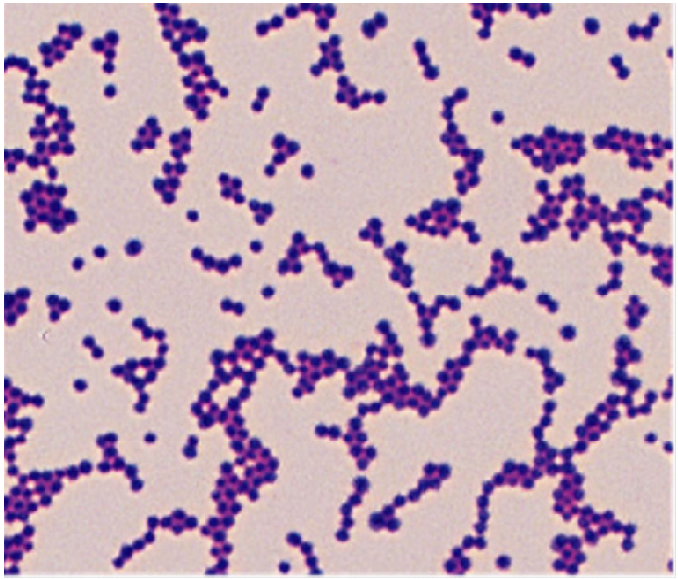
- Bacterial Smear
- Methylene bluye stain for 1 minute
- Rinse in Water
- Blot & Dry
- Oil Immersion Objective

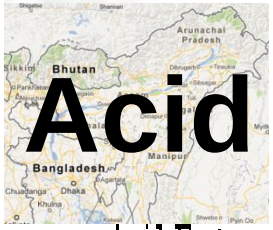


Gram's Staining

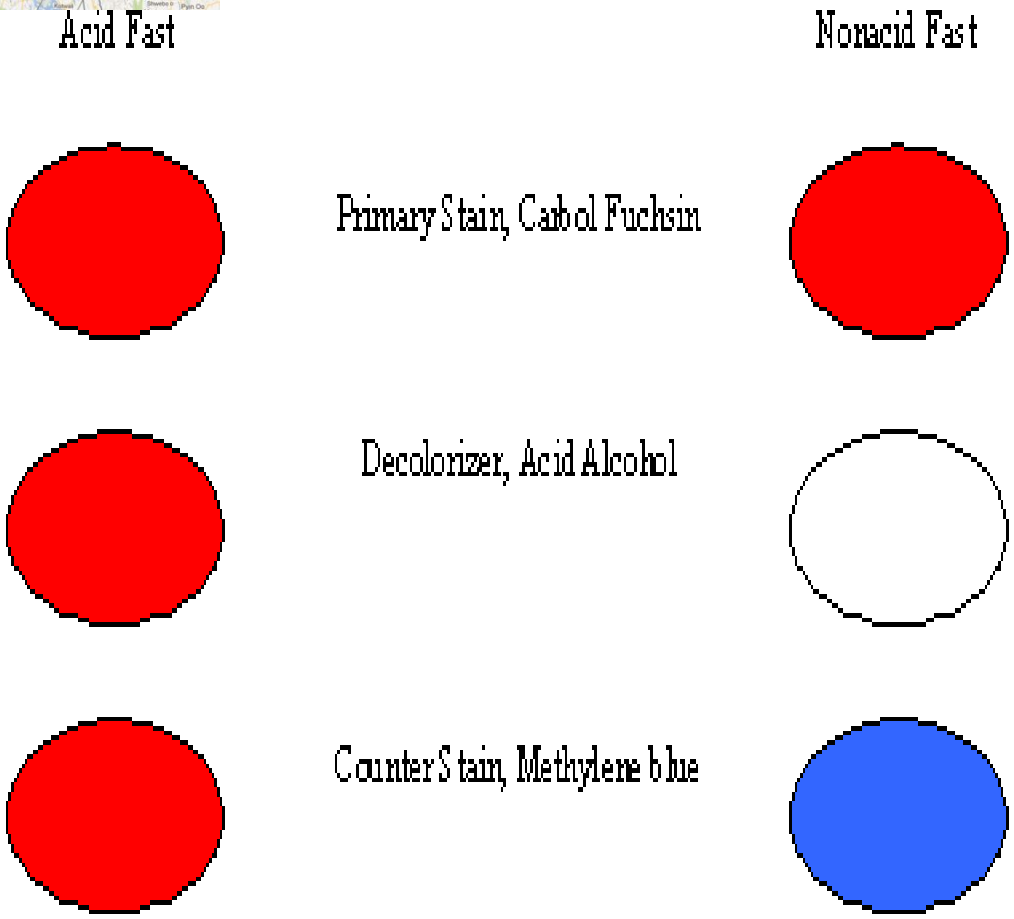


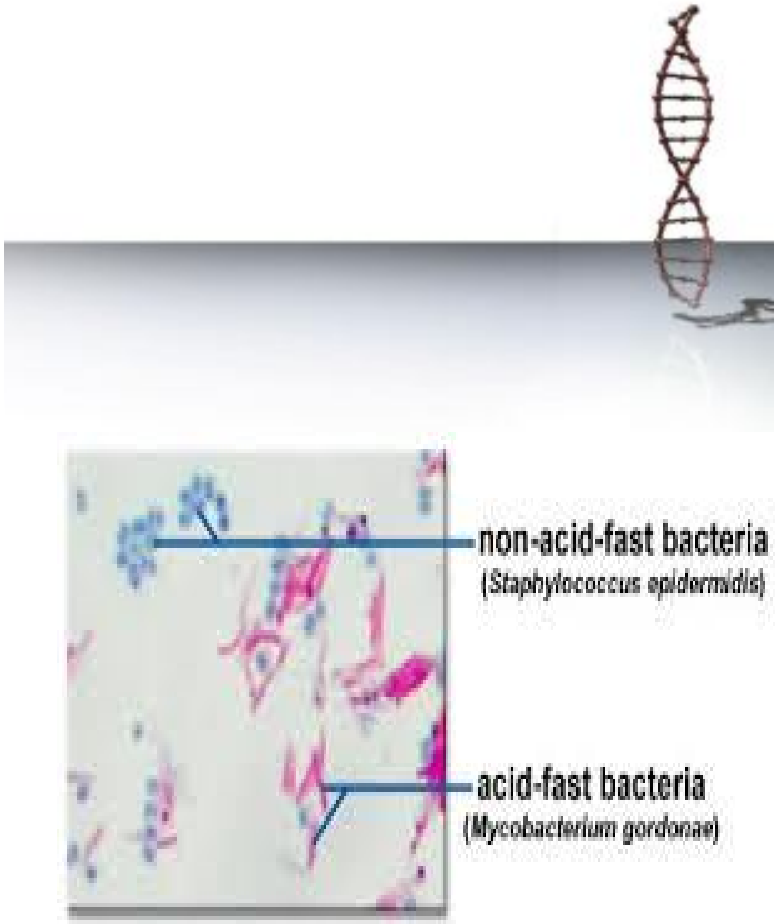






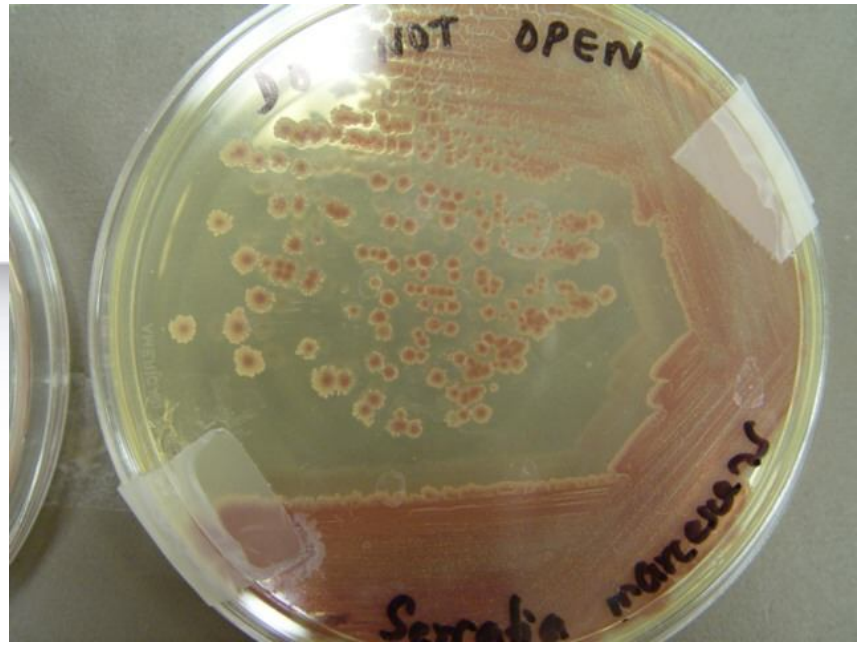
Acid Fast Staining

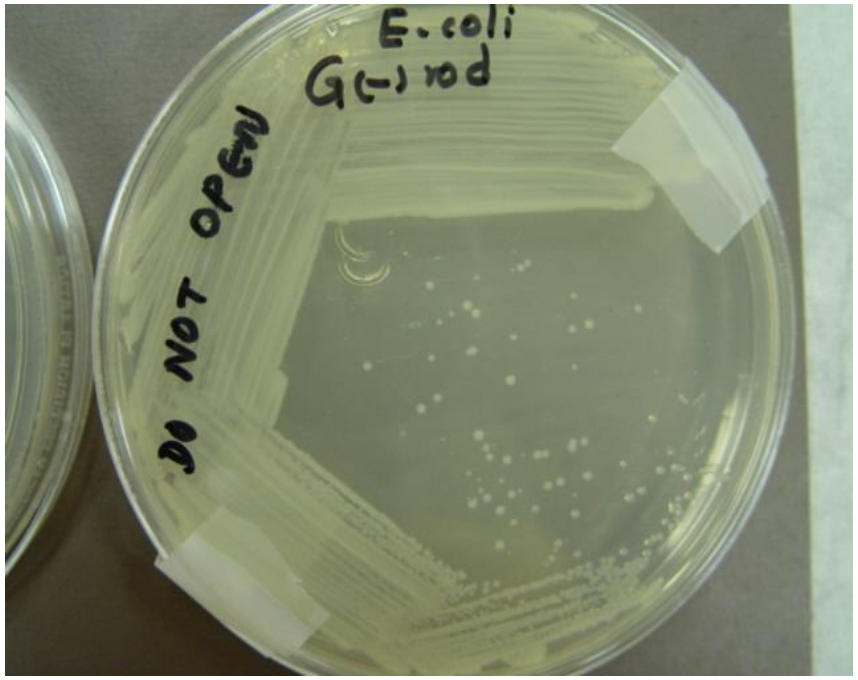




Requirements- For Cultural Examination

Sl. No	Materials	Source
1	Inoculating loop	Tarsons HIMedia Borosil SISCO Research Lab
2	Petri dish	
3	Spirit Lamp/ Gas burner	
4	Scissor and Forceps	
5	Media Nutrient broth Nutrient Agar MLA Blood Agar Selenite broth	
6	Conical Flask, Test tube, Swab	
7	Measuring Cylinder	
8	Electronic Balance & Autoclave	





Requirements- For Antibiogram

Sl. No	Materials	Source
1	Antibiotic Disc	Tarsons HIMedia Borosil SISCO Research Lab
2	Forceps	
3	Petri dish	
4	Test tube & Rack	
5	Sterile Cotton Swab	
6	Media- Nutrient broth & Nutrient Agar	
7	Spirit Lamp/Gas burner	

Requirements- For RBPT

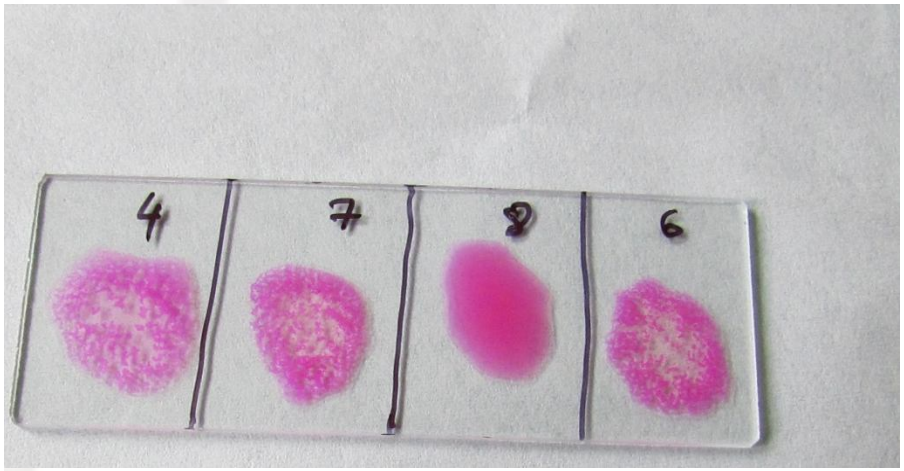
Sl. No	Materials	Source
1	Micro slide	Tarsons HIMedia Borosil SISCO Research Lab
2	Rose Bengal Antigen (Source: IVRI or State Biologicals)	
3	Test Serum	
4	Micro pipette & Tips	
5	Glass/Plastic rod	

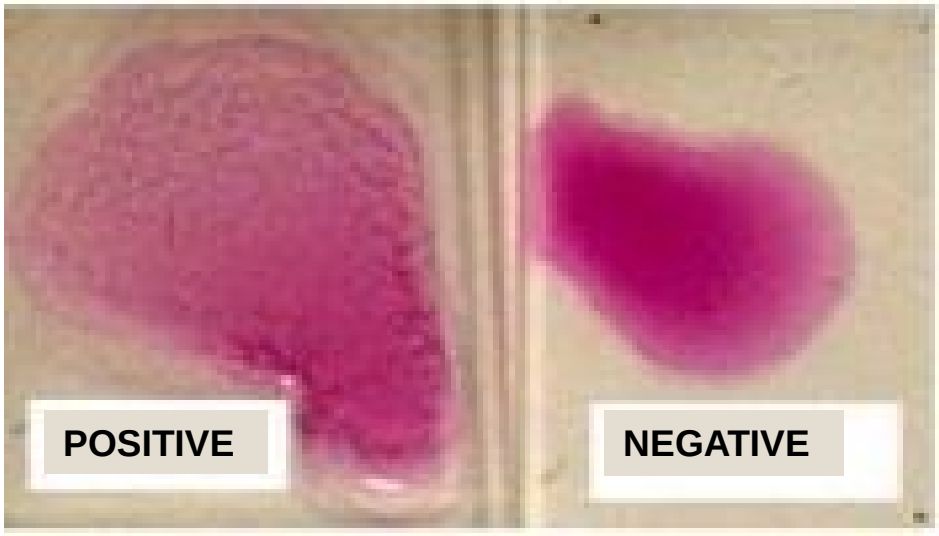
RBPT Procedure

- Bring the serum samples and antigen to room temperature (22 ± 4°C)
- Place 25–30 µl of each serum sample on a white tile or glass slide
- Place an equal volume of antigen near each serum spot
- Mix the serum and antigen thoroughly (using a clean glass or plastic rod for each test) to produce a circular or oval zone approximately 2 cm in diameter.
- Read for agglutination immediately after the 4- minute period is completed

Rose Bengal Plate Test (RBPT) for Brucellosis

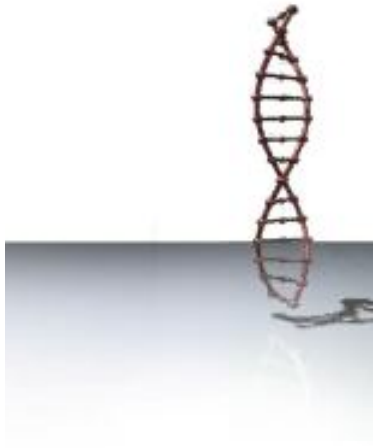
- Rose Bengal antigen interacts with serum antibody to produce agglutination, which is used for the detection of Brucella specific antibodies

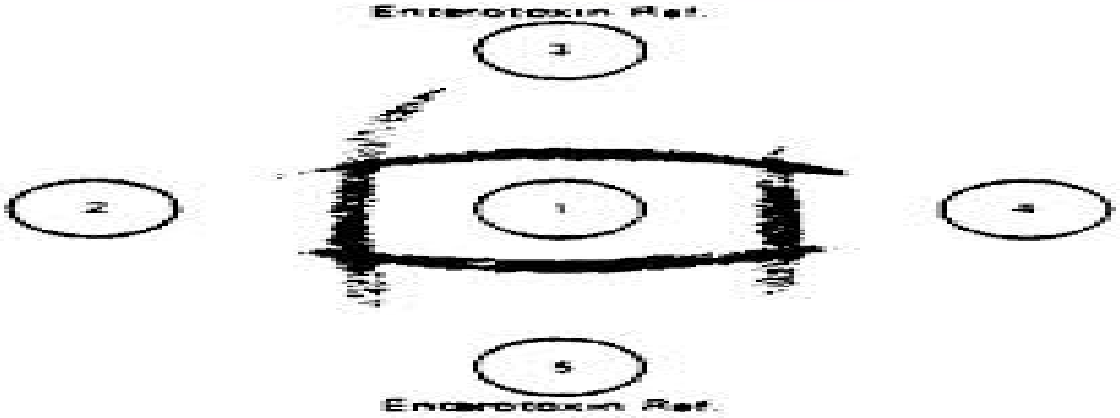


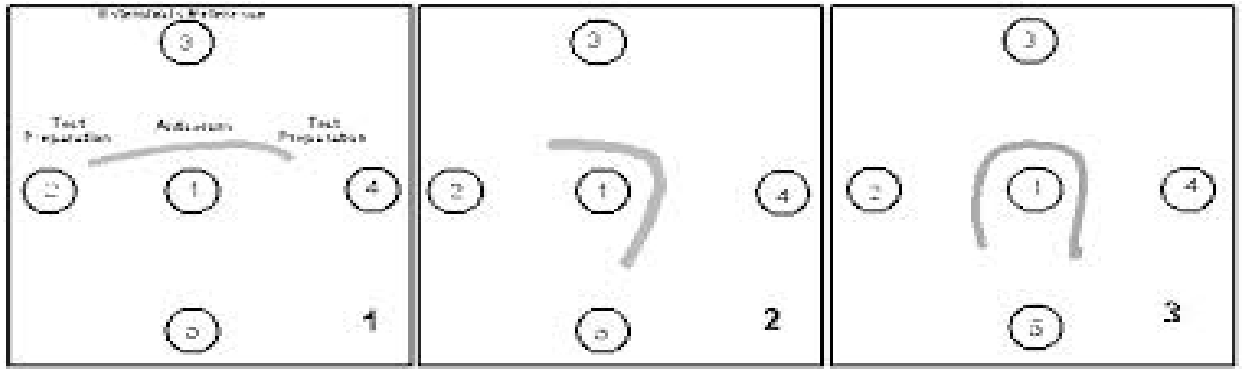


Requirements- For AGPT

Sl. No	Materials	Source
1	Micro slide	Tarsons HIMedia Borosil SISCO Research Lab
2	Agarose	
3	Pipette	
4	Gel Puncher & Needle	
5	Micro Pipette & Tips	
6	Hyper immune sera	

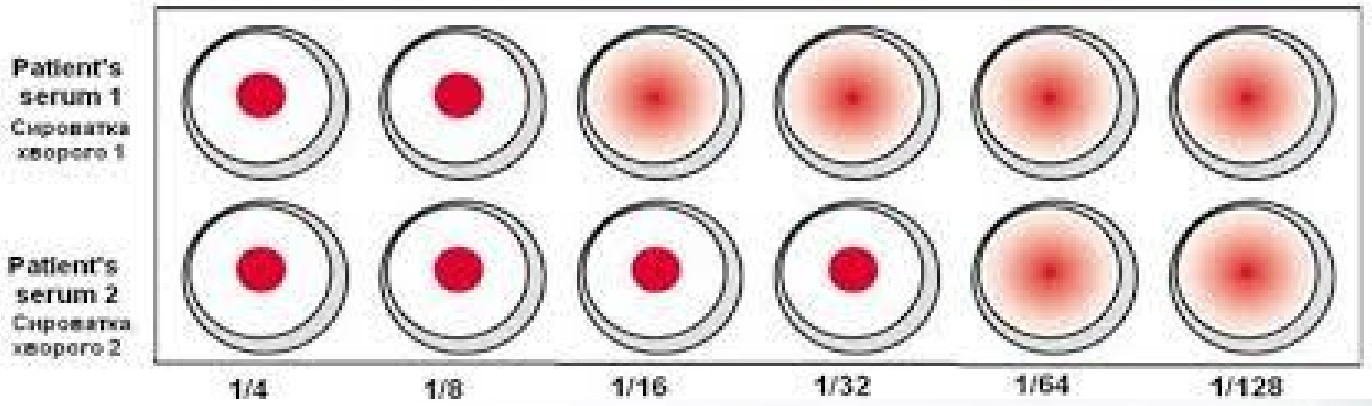




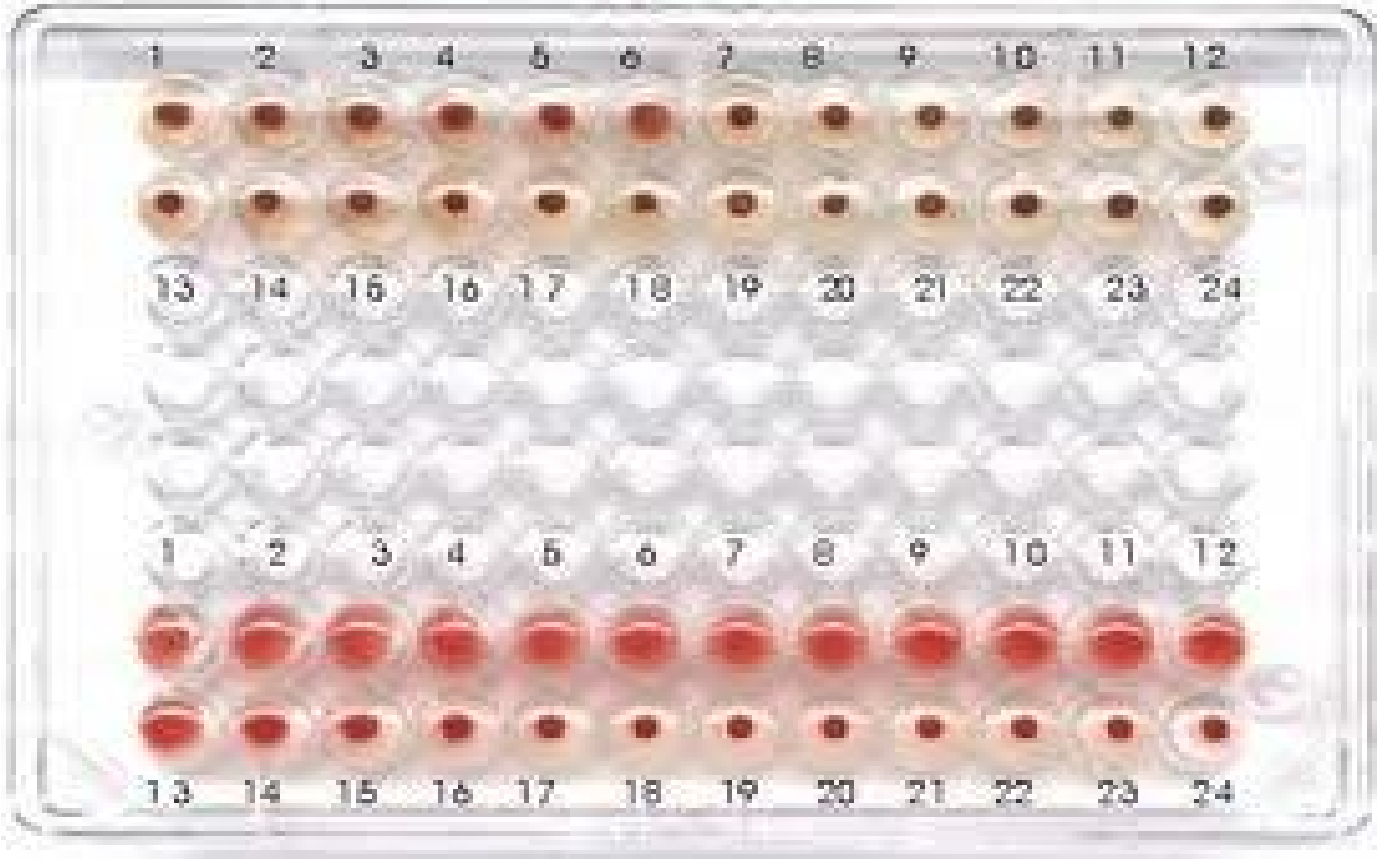


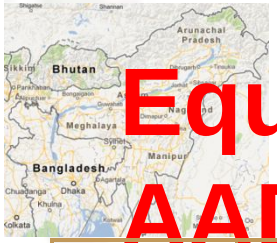
Requirements- For HA/HI

Sl. No	Materials	Source
1	Centrifuge Machine	Tarsons HIMedia Borosil SISCO Research Lab
2	Centrifuge tube	
3	96 well U shaped micro-titre plate	
4	Micro Pipette & Tips	
5	Pipette, Conical flask	
6	Measuring Cylinder	
7	Chicken RBC	
8	Alsever's Solution	
9	Water Bath	
10	PBS (P^H 7.2-7.4)	



Results of HA/HI





Equipments to be availed under AADSMC

Sl. No	Description of Equipments	Grant Recommended
1	Dual refrigerator with Stabilizer (4X3=12)	5.40
2	Compound Microscope (4X3=12)	4.14
3	Table top centrifuge machine (4X3=12)	4.80
4	BOD Incubator (4x3=12)	16.80
5	Hot Air Oven (4X3=12)	9.60
6	Autoclave (4X3=12)	4.80
7	Micropipette Set (4X3=12)	9.42
8	Post Mortem Set (4X3=12)	1.20
9	Vaccine Carrier (4X2X3=24)	0.72

Budget (For 3 State):

S. N.	Head	1st Yr	2nd Yr	3rd Yr	4th Yr	5th Yr	Total
A. Non-Recurring							
1	Equipment & accessories	0.00	56.88	0.00	0.00	0.00	56.88
	Strengthening of lab infrastructure	0.00	45.48	45.48	45.48	45.48	181.92
	Total (A)	0.00	102.36	45.48	45.48	45.48	238.80
B. Recurring							
2	Travel	0.00	3.00	3.00	3.00	3.00	12.00
3	Contingency	0.00	3.00	3.00	3.00	3.00	12.00
4	Overhead Charges	0.00	1.50	1.50	1.50	1.50	6.00
	Total (B)	0.00	7.50	7.50	7.50	7.50	30.00
	Grand Total	0.00	109.86	52.98	52.98	52.98	268.80

THANK YOU

Application of GIS systems in disease monitoring

Arnab Sen

ICAR Research Complex for NEH, Barapani

Learning Objectives

- Develop awareness of the basic capabilities of Geographic Information Systems in Public Health
- Understand basic concepts associated with GIS software
- Understand specific ways in which GIS can be used in Public Health

Learning Objectives

- Develop familiarity with terminology related to GIS
- Understand issues related to privacy and confidentiality of records using in GIS software and maps which are produced by GIS software

Performance Objectives

- Determine whether a particular study would benefit from GIS as a tool.
- Determine the need for a data security method to be applied to a published map created with GIS software.

GIS Terminology

- **Geocoding**
  - Append latitude and longitude to an address
- **Centroid**
  - Center of a region
- **Layer**
  - One type of geography on a map

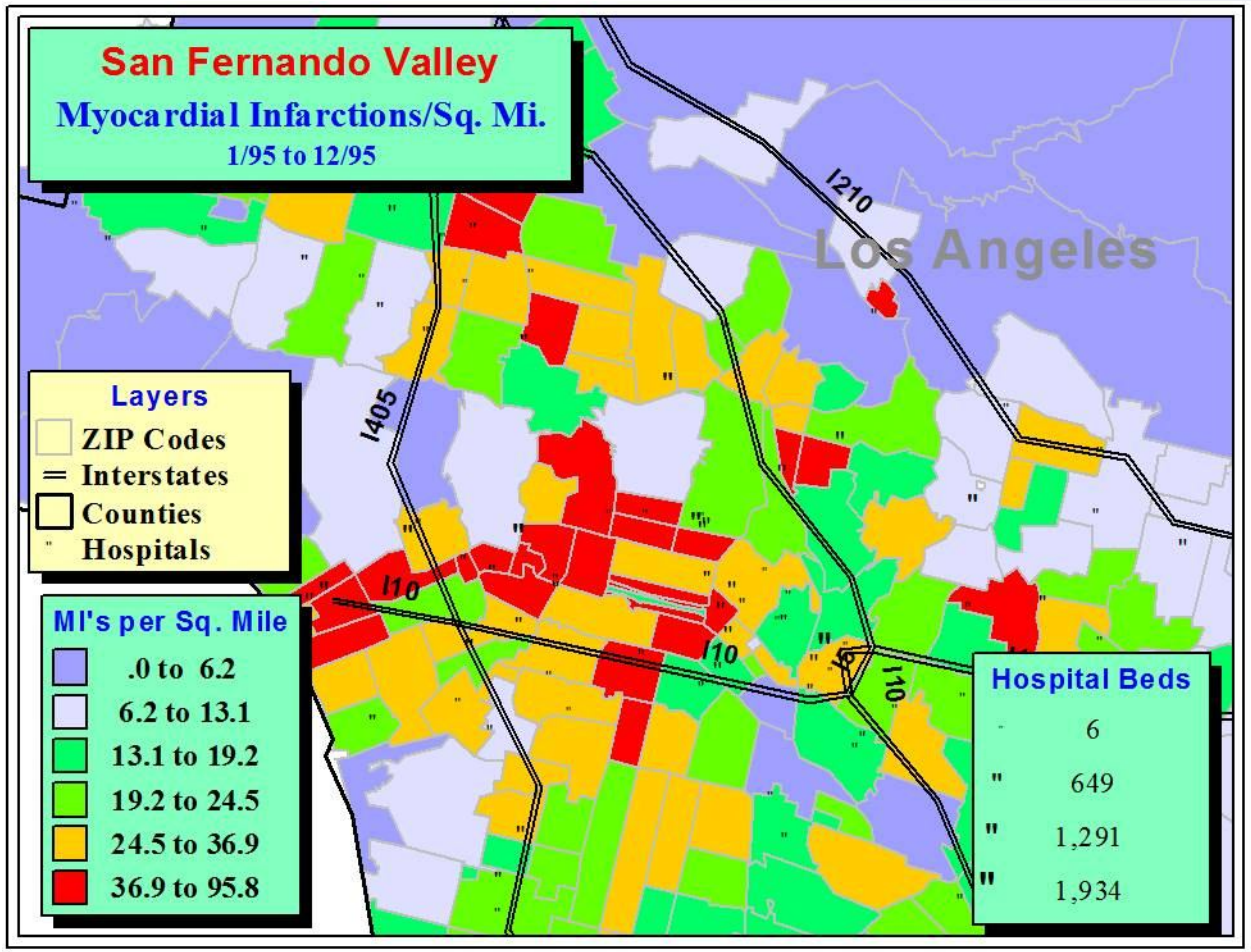
GIS Terminology

- **Features**
  - –Anything on a map
- **Attributes**
  - –Characteristics of features
- **Thematic mapping**
  - –Visualization of feature attributes

GIS Concepts

- Types of geography
  - –Regions (polygons)
  - –Points
  - –Lines

Example of a Theme Map

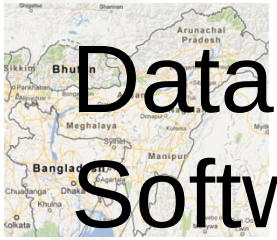


GIS Terminology

- Projections.
  - –Compensate for curvature of the earth.

Types of Graphic Images

- **Raster**
  - **Made up of pixels**
  - **No relationship between elements**
- **Vector**
  - **Points connected by lines**



Data Requirements for Use of GIS Software

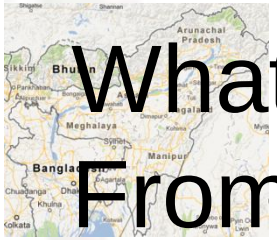
- Data must be organized geographically
- Records must include addresses or,
- Records must include the region the point is located in (such as a zip code, census tract, etc.)

Types of Applications in Public Health

- Analytical
- Reporting
- Data transformation
- Spatial Database Queries

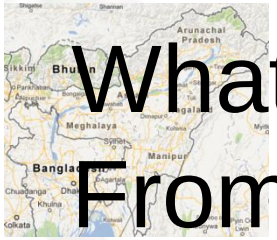
Examples of Spatial Database Queries

- Identifying and locating disease clusters
- Locating target populations
- Identifying and locating community resources useful for intervention programs



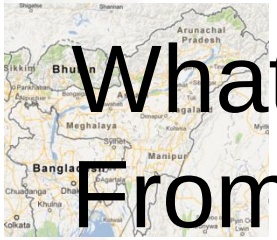
What Types of Studies Can Benefit From a GIS?

- Calculation and visualization of morbidity and mortality rates for regions
- Calculation of distance variables for statistical testing



What Types of Studies Can Benefit From a GIS?

- Data visualization from a spatial perspective
  - –Identifying geographic patterns
- Risk communication
  - –Communicating geographically related risks



What Types of Studies Can Benefit From a GIS?

- Disease cluster analysis
  - Locating larger than expected incidences of a disease related by time and place

Analytical Barriers to Cluster Analysis

- Imprecise measure of time
- Imprecise measure of place
- Incomplete clinical data

Disease Cluster Analysis

- For there to be causation, the risk must:
  - –Be in geographical proximity to the case, and…
  - –Pre-date the disease

Privacy and Confidentiality Issues

- **Problem**: maps may contain sufficient detail so that individual cases can be identified.
- Primarily an issue when maps are released to the public or included in published work.

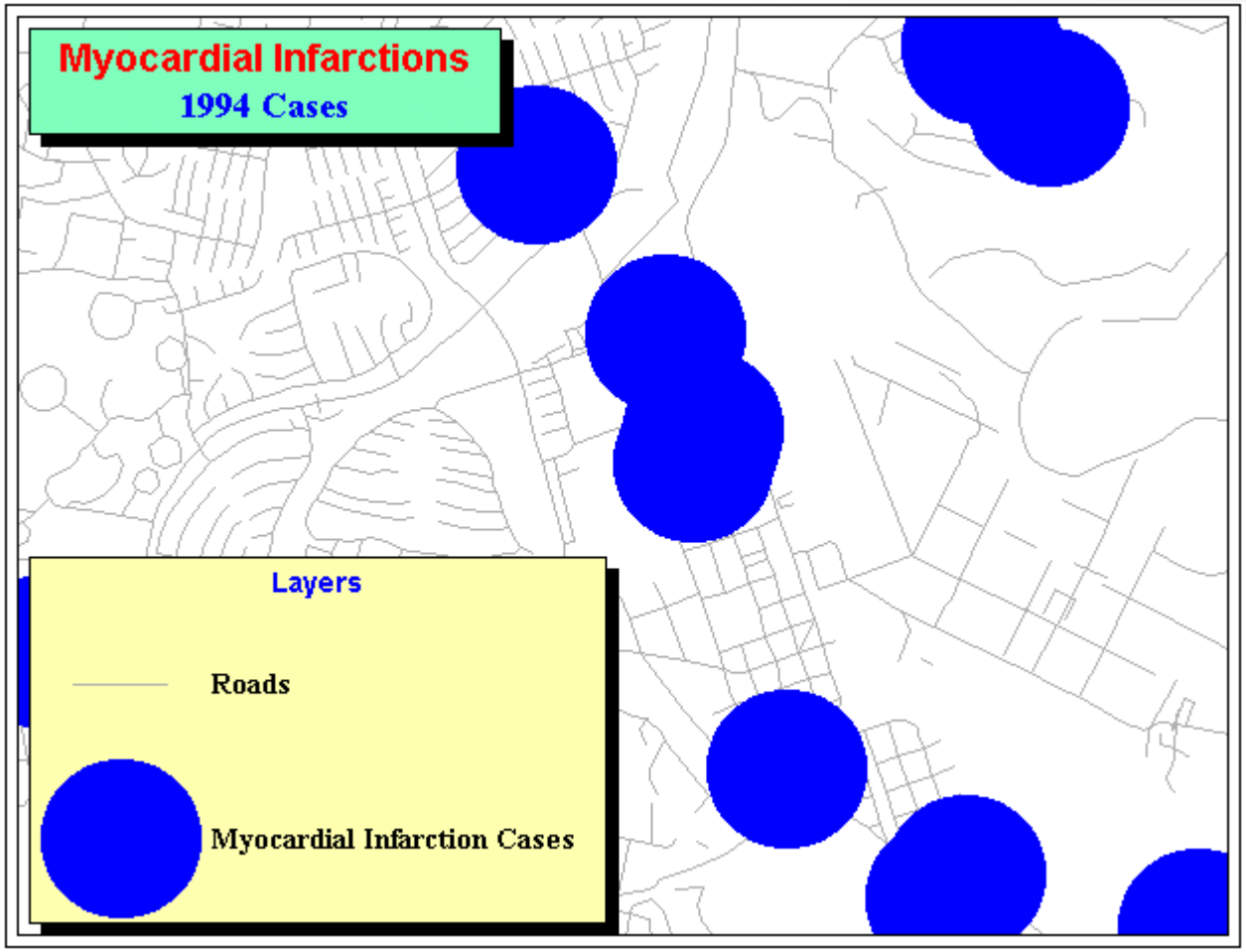
Methods to Protect Confidentiality

- **Introduction of Error**
  - –Points are randomly dispersed
- **Limitation of Detail**
  - –Detailed layers, such as streets are not displayed with points.

Methods to Protect Confidentiality

- Suppression of records
  - Records which meet very limited criteria and are few in numbers are not shown or disguised
- Large Symbols

Example of Large Symbol Method



Methods to Protect Confidentiality

- Convert points into attributes.
  - Rather than display points, the points are aggregated to regions and the regions are themed by color based on the the number of points they contain.

What is a geographic information system?

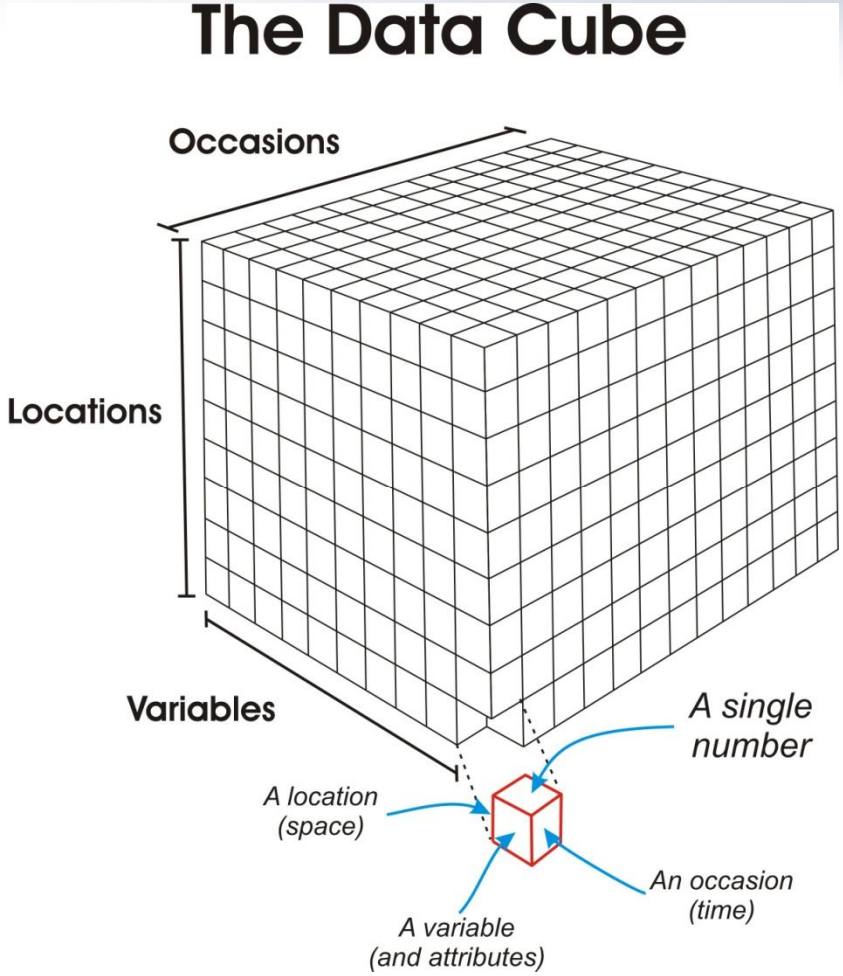
- A computer system designed for storing, manipulating, analyzing, and displaying data in a geographic context.
- a computer system designed to allow users to collect, manage and analyze large volumes of spatially referenced information and associated attribute data.
- Analysis that combine relational databases with spatial interpretation and outputs often in form of maps.
- A GIS represents within a computer real-world geographic relationships and allows them to be analyzed and modeled.

Topology is at the heart of GIS analysis

- The geometric characteristics of objects which do not change under transformations (stretching or bending) and are independent of any coordinate system.
- The topological characteristics of an object are also independent of scale of measurement.
- Topology as it relates to spatial data consists of three elements:
  - Adjacency.
  - Containment.
  - Connectivity.
- In GIS, distance, area, volume can be measured.
- Almost anything can be mapped.

Questions that can be answered with a GIS

- What is there?
- How much is there?
- Why isn't it elsewhere?
- Was it there in the past?
- Will it be there in the future?



GIS research can be basic or applied

- Basic research.
  - Modeling land use change.
  - Spread of a disease through a population.
  - Impact of global climate change on agriculture.
  - Determining an endangered species' habitat.
  - The ideal location of health centers to equally provide service to all people in a country or region.
  - Modeling urban growth.
  - Previous geographic states: pre-crustal (isostatic) rebound Michigan.

GIS research can be basic or applied

- Applied research.
  - Environmental protection/restoration.
  - Pollution mapping and modeling.
  - Crime mapping.
  - Natural resource management.
  - Power allocation by utilities companies.
  - Most efficient routing for vehicles.
  - Marketing areas.
  - Surveillance of disease outbreak and spread.
  - Planning urban growth.
  - Facilities (gas lines, fire hydrants, etc.) management.
  - County and city property taxation.

Two principal types of GIS, raster and vector

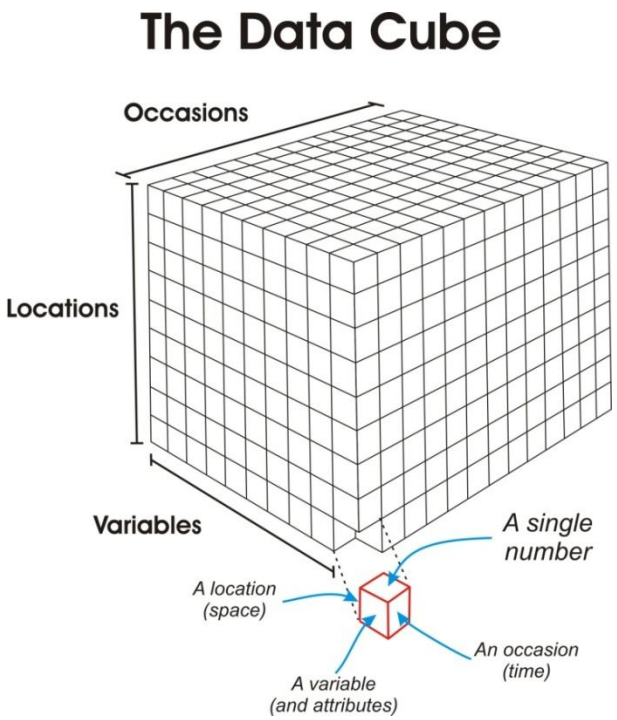
- Raster GIS.
  - Raster representations divide the world into arrays of cells.
  - Geographic unit of raster GIS.
    - The cell (pixel).
    - A unique reference coordinate represents each pixel either at a corner or the centroid.
  - Attributes (variables) are assigned to the cells.

Two principal types of GIS, raster and vector

- Vector GIS.
  - Displays and defines features on the basis of two-dimensional Cartesian coordinate pairs (*x* and y) and computational algorithms of the coordinates.
  - Geographic elements in vector GIS:
    - Point = node.
    - Line = vertex or arc.
    - Area = polygon.
  - Each element can be given attributes.
    - Location.
    - Adjacency.
    - Variables.

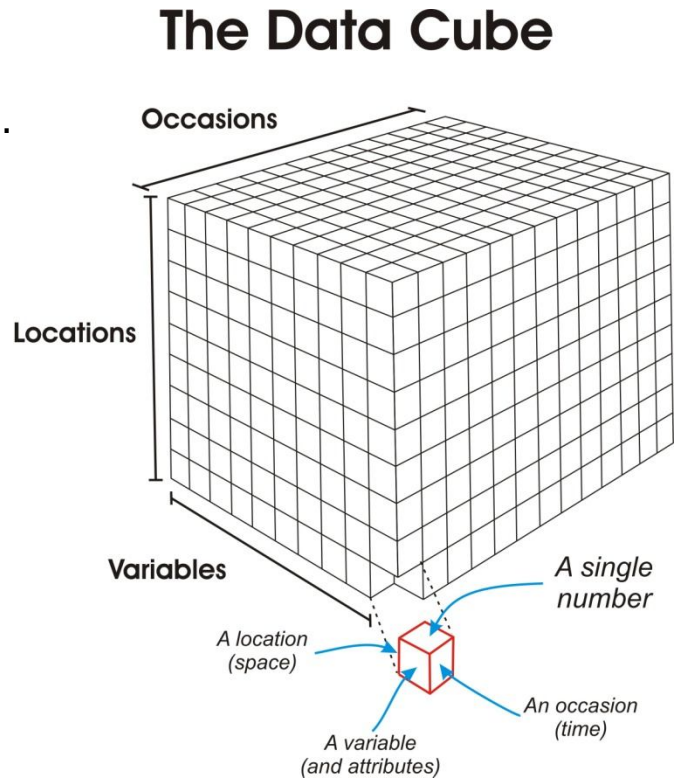
Two types of GIS data, attribute and spatial

- Attribute data.
  - A characteristic of a feature that contains a measurement or value for the feature.
  - The variable being measured.
  - An item for which data are collected and organized.
    - Labels.
    - Categories.
    - Numbers.
    - Dates.
    - Standardized values.
    - Field or other measurements.
  - Can be at any level of measurement.
    - Nominal.
    - Ordinal.
    - Interval/ratio.



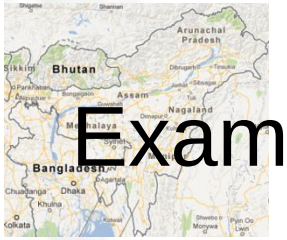
Two types of GIS data, attribute and spatial

- Spatial data.
  - Geographical location.
  - Where the variable being measured is located.
    - Points.
    - Lines.
    - Polygons.
    - Pixels.



Comparison of raster and vector

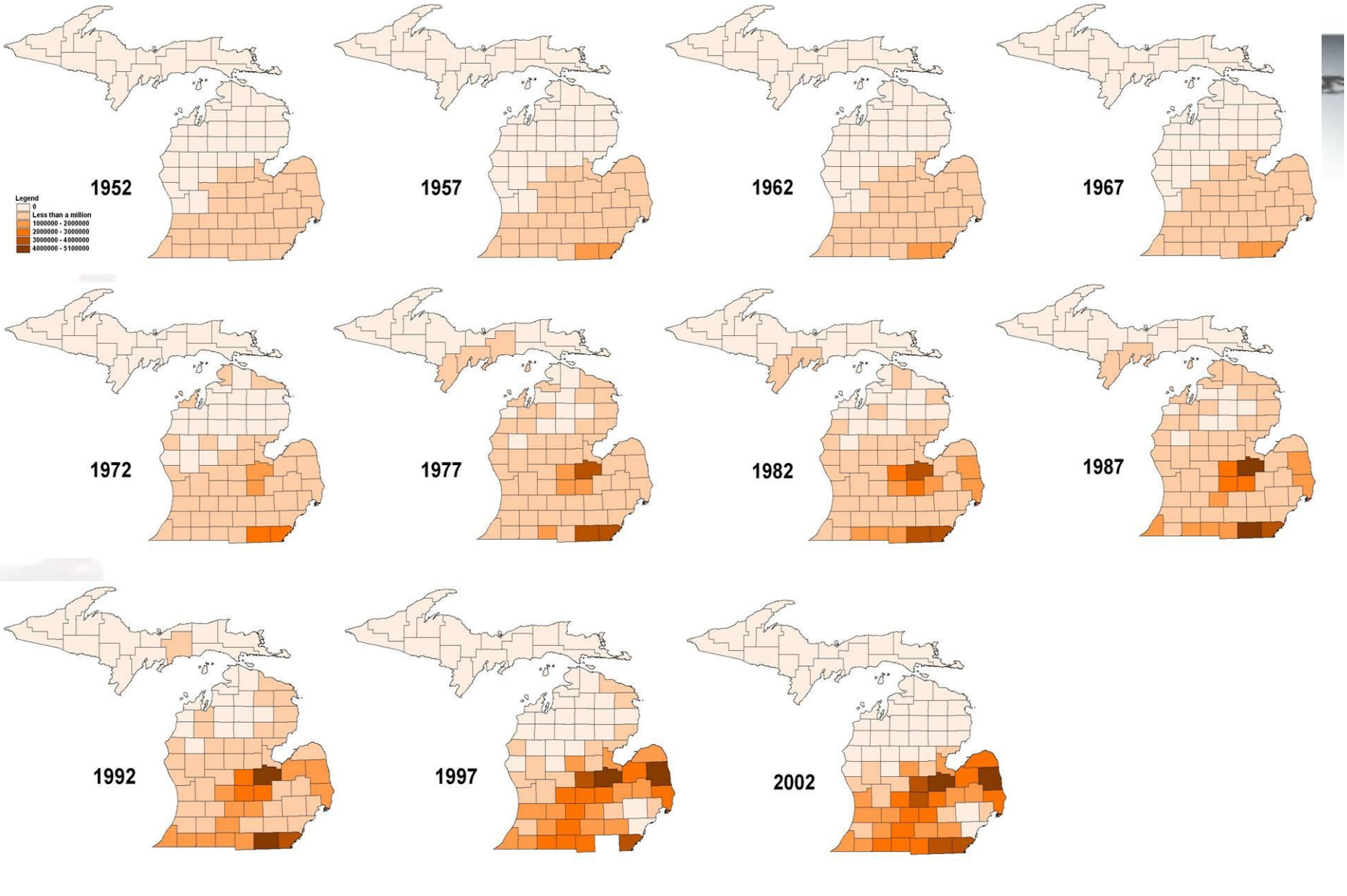
Method	Advantages	Disadvantages
Raster	Simple data structure	Requires greater storage space on computer
	Compatible with remotely sensed or scanned data	Depending on pixel size, graphical output may be blocky and "unmaplike"
	Simple spatial analysis procedures	Projection transformations are more difficult
		More difficult to represent topological relationships
Vector	Requires less disk storage space	More complex data structure
	Topological relationships are readily maintained	Not as compatible with remotely sensed data
	Graphical output more closely resembles hand-drawn maps	Software and hardware are often more expensive
		Some spatial analysis procedures may be more difficult
		Overlaying multiple vector maps is often time consuming



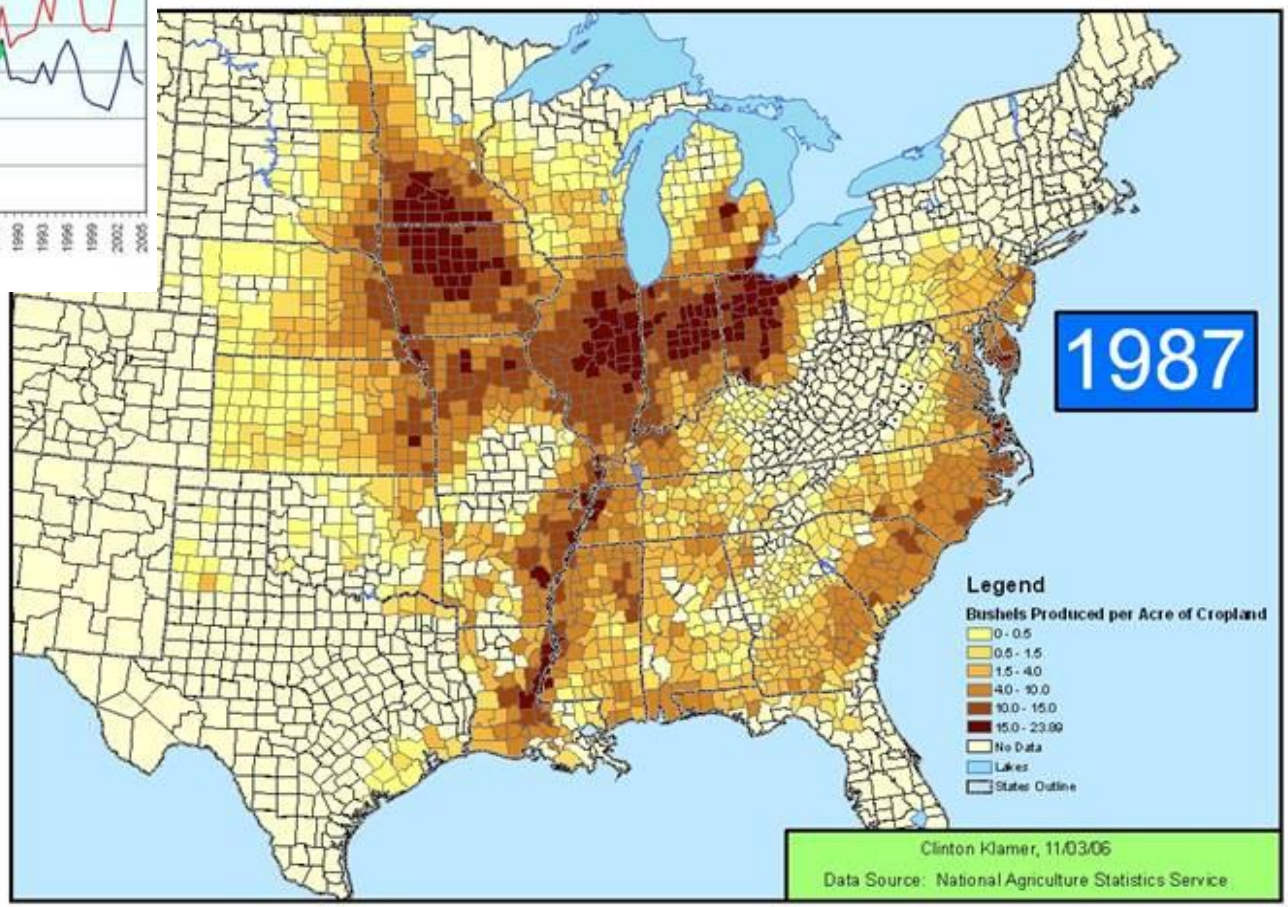
Examples

- How many people live within 10 miles of a superfund site in Michigan?
- Sales by market region by sales agent.
- Population change in any state over time.
- Spread of soybean cultivation across the United States.
- What is the best place to site the Grand Haven bypass?
- Prolonged drought and land use change over a 33-year period in central Mali, West Africa.
- Does living next to light rail public transportation increase the value of property?
- Where are the pediatricians who are chosen by women who give birth at North Ottawa Community Hospital located: Grand Haven or elsewhere?

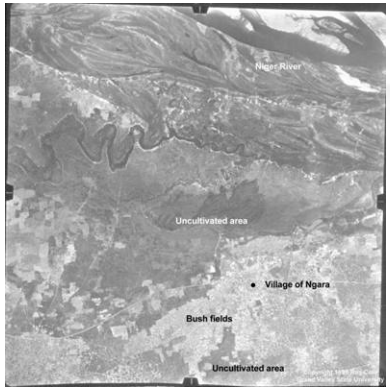
Change in soybean production in Michigan, 1952 to 2002

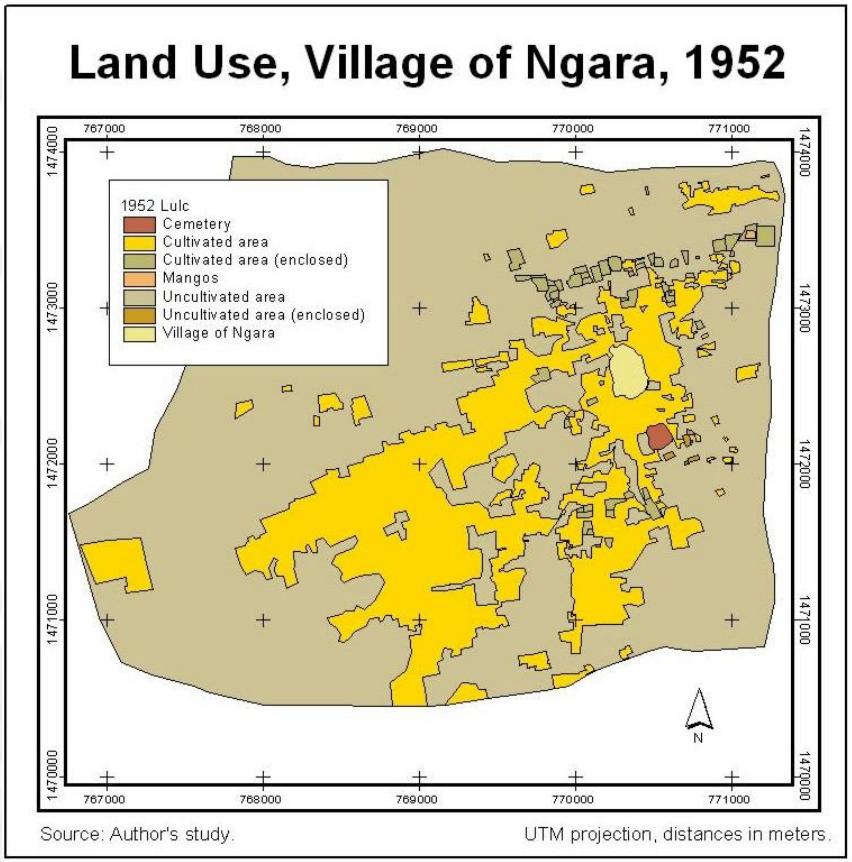


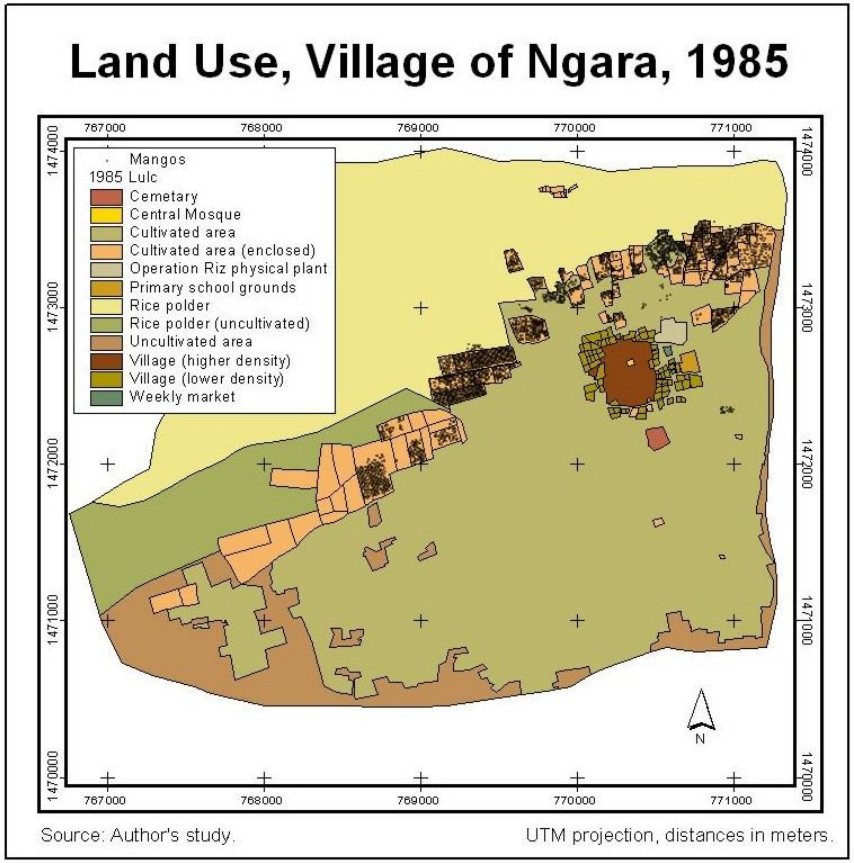
Diffusion of Soybean Cultivation, 1927 to 2005



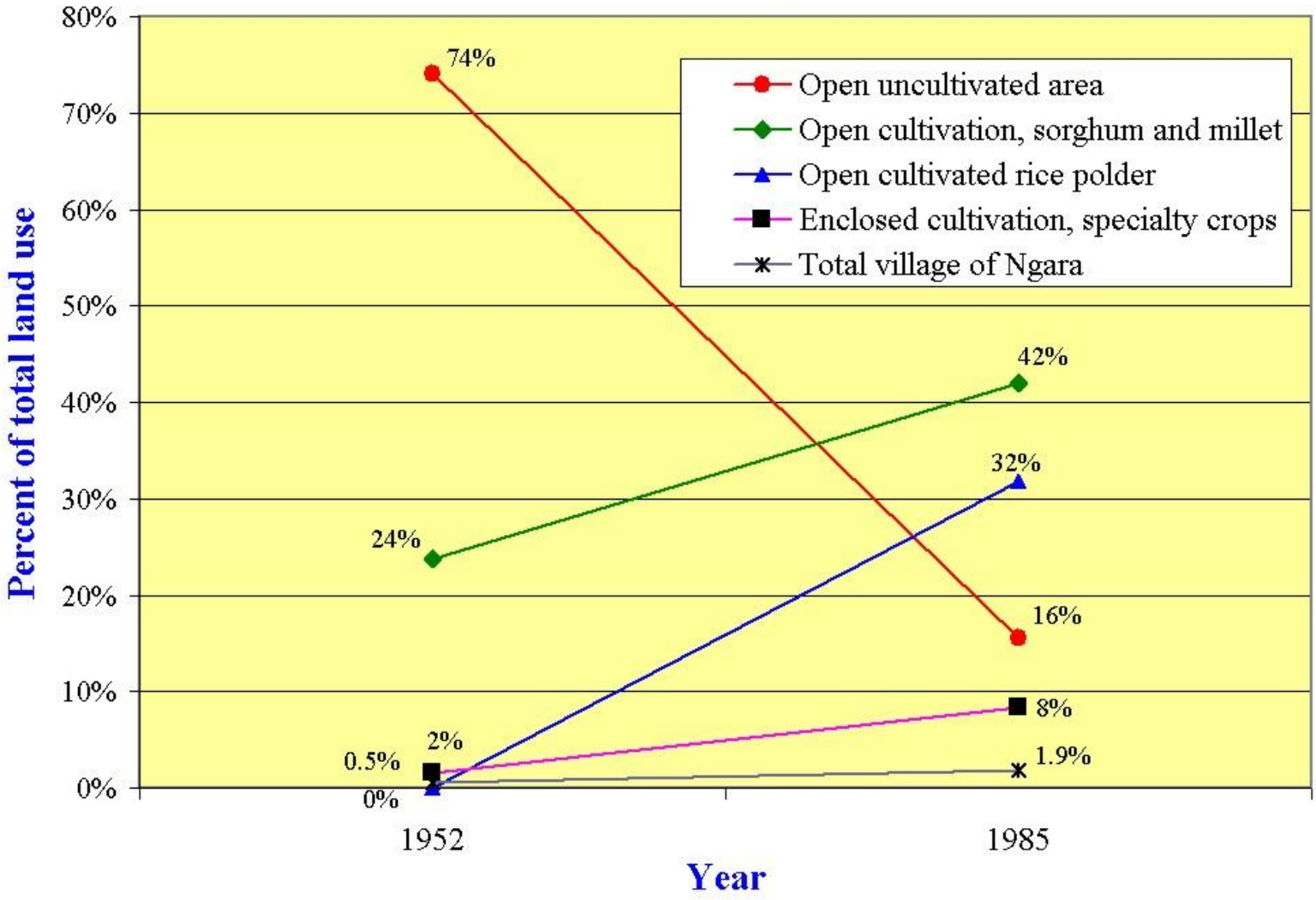
GIS is useful to understand land use change in West Africa



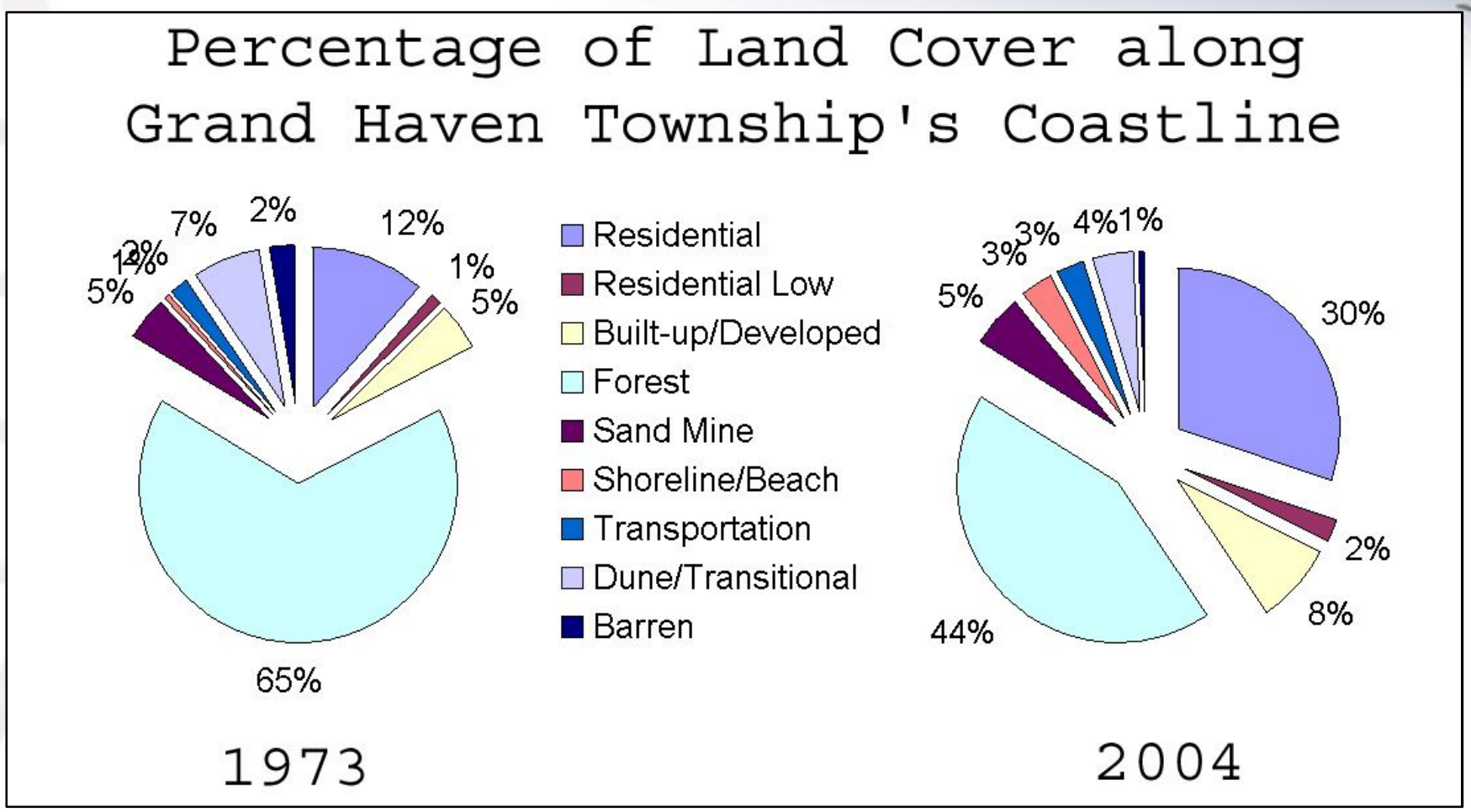




Land Use Class as Percent of Total Land Use, 1952 and 1985, Ngara

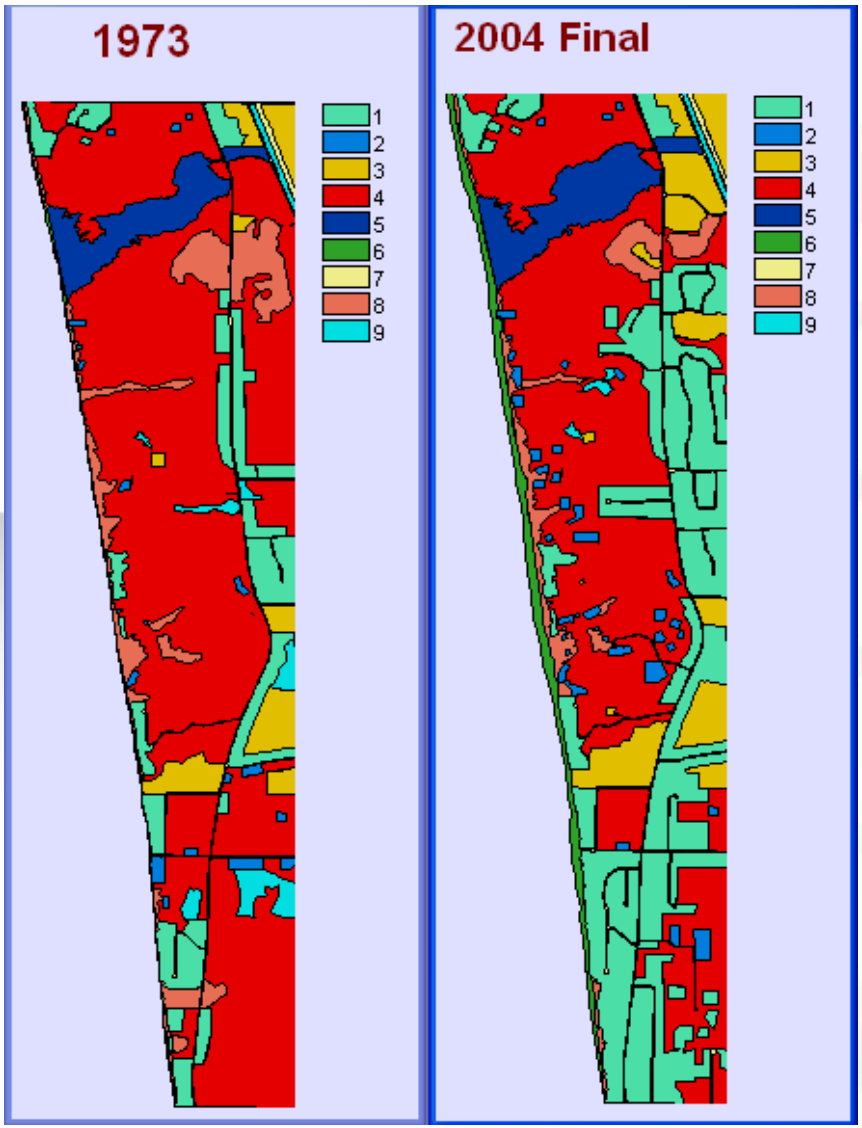


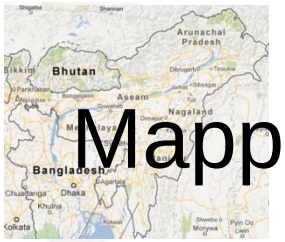
Mapping land use/cover change along Lake Michigan in Grand Haven Township, 1973 to 2004



Mapping land use/cover change along Lake Michigan in Grand Haven Township, 1973 to 2004

Land Cover	1973 Area
Residential	223.641
Residential Low	20.726
Built-up/Developed	94.223
Forest	1273.658
Sand Mine	89.838
Shoreline/Beach	9.812
Transportation	35.672
Dune/Transitional	135.491
Barren	48.023





Mapping crime

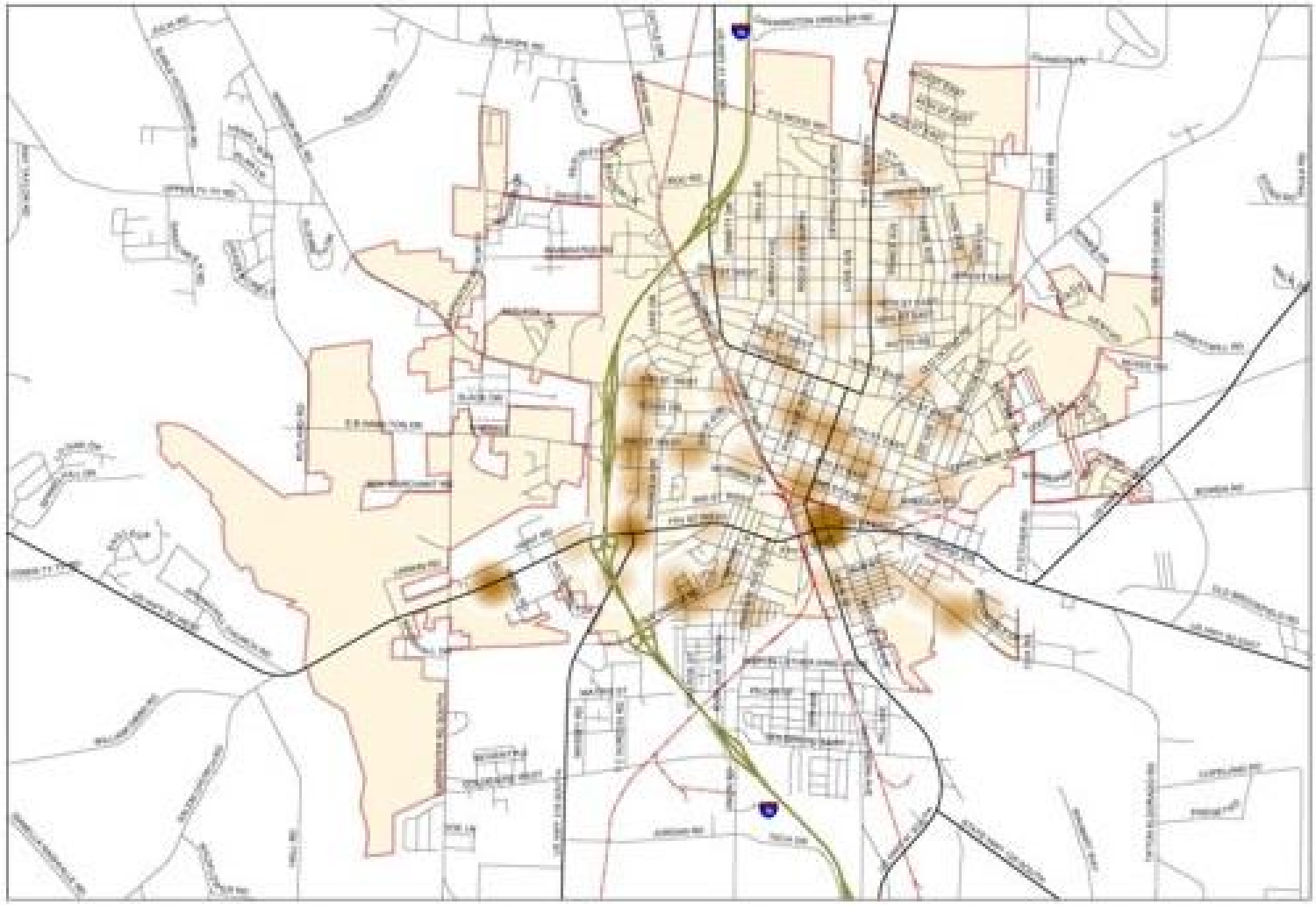
- Identifying priority neighborhoods (Vulnerable Localities Index).
- Geographical indicators of community cohesion.
- Crime hotspots.
- Profiling hot spots.
- Predicting patterns of criminal activity.

Crime mapping, Tifton, Georgia

- Beginning in 2004, the South Georgia Regional Development Center began geocoding crime incidents for Tifton, Georgia (population 15,000).
- Officials wanted to see how the crime patterns would look.
- Initially, a density map for all Part I crimes for the period of February 2004–April 2004 was made.
- After that, crimes were broken down by six-hour time periods (midnight to 6:00 a.m., 6:00 a.m. to noon, noon to 6:00 p.m., 6:00 p.m. to midnight) to see how density patterns would vary by time of day.
- Analyzing crime incidents by time of day helps law enforcement concentrate patrol efforts during specific time periods.

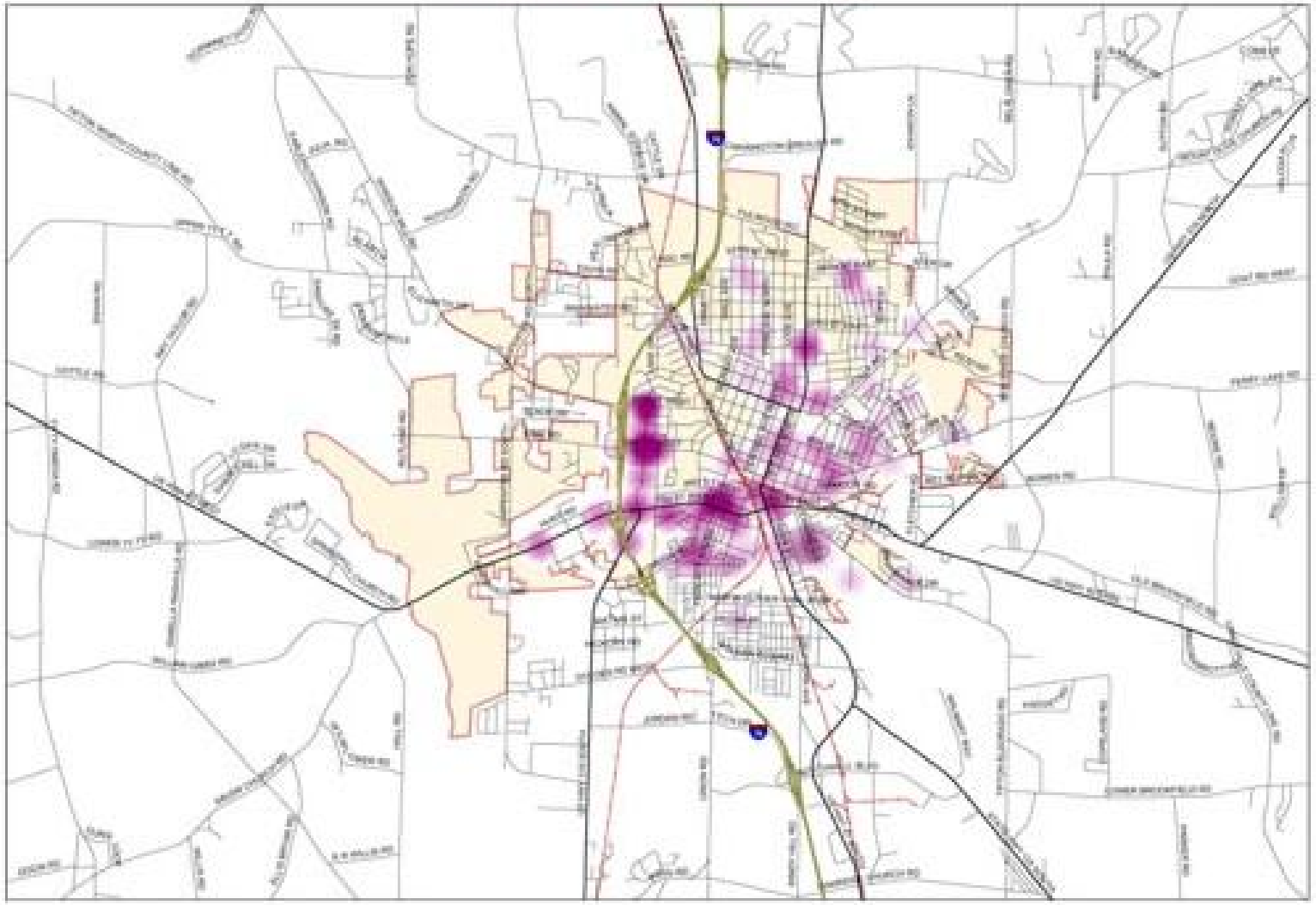
Crimes between 6AM and 12PM

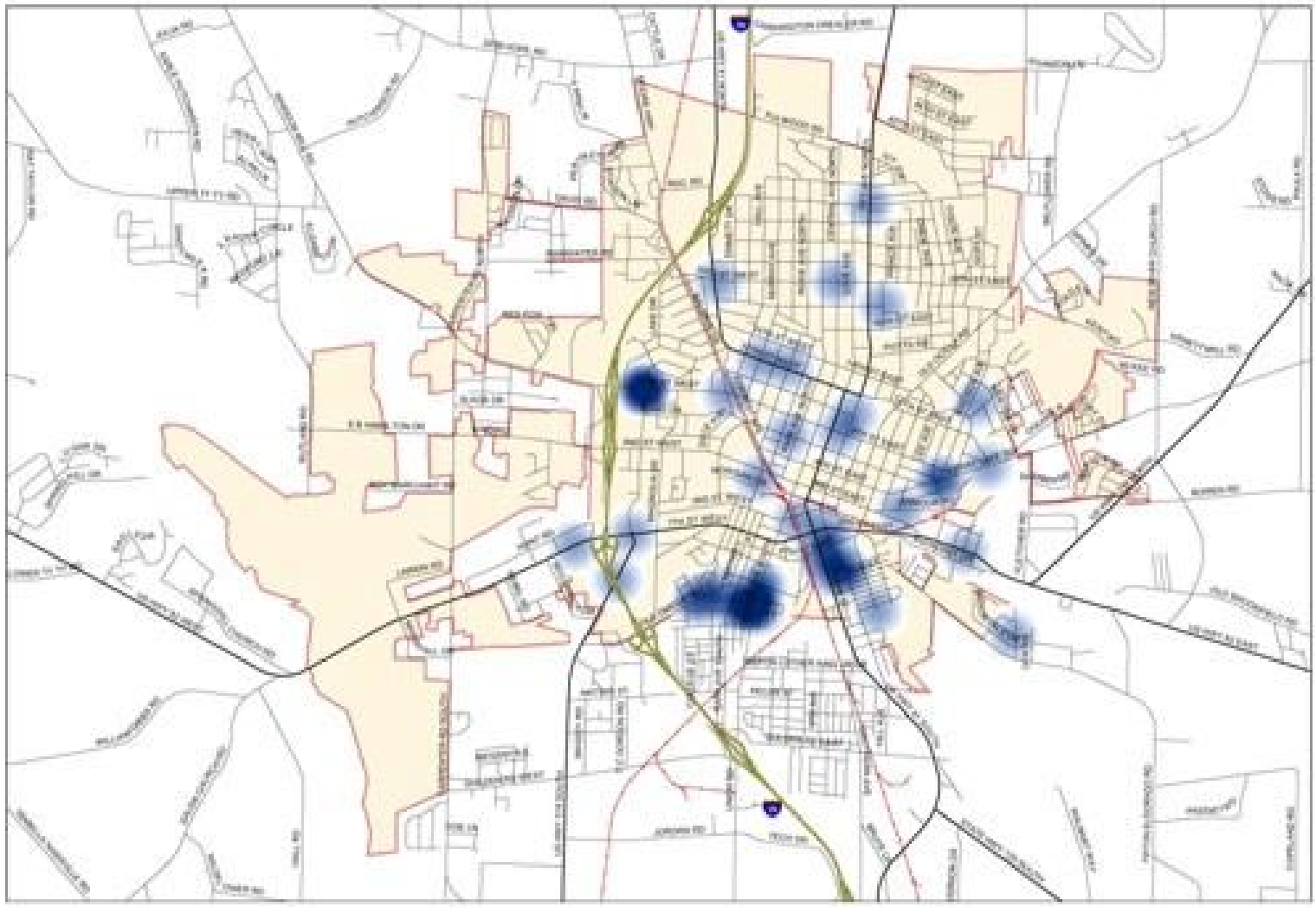
Total Number of Crimes = 62



Crimes between 6PM and 12AM

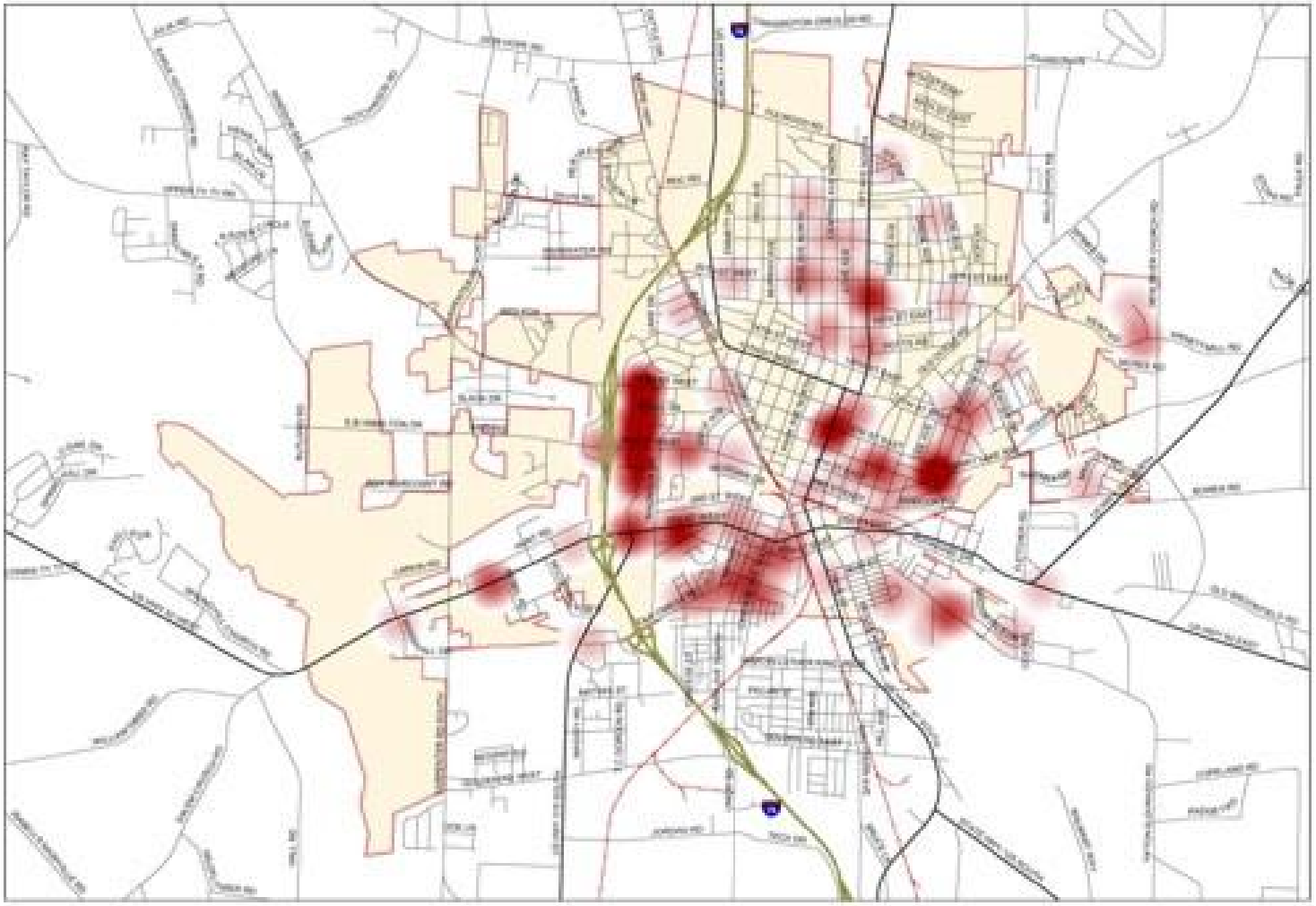
Total Number of Crimes = 143





Crimes between 12AM and 6AM

Total Number of Crimes = 43



Crimes between 12PM and 6PM

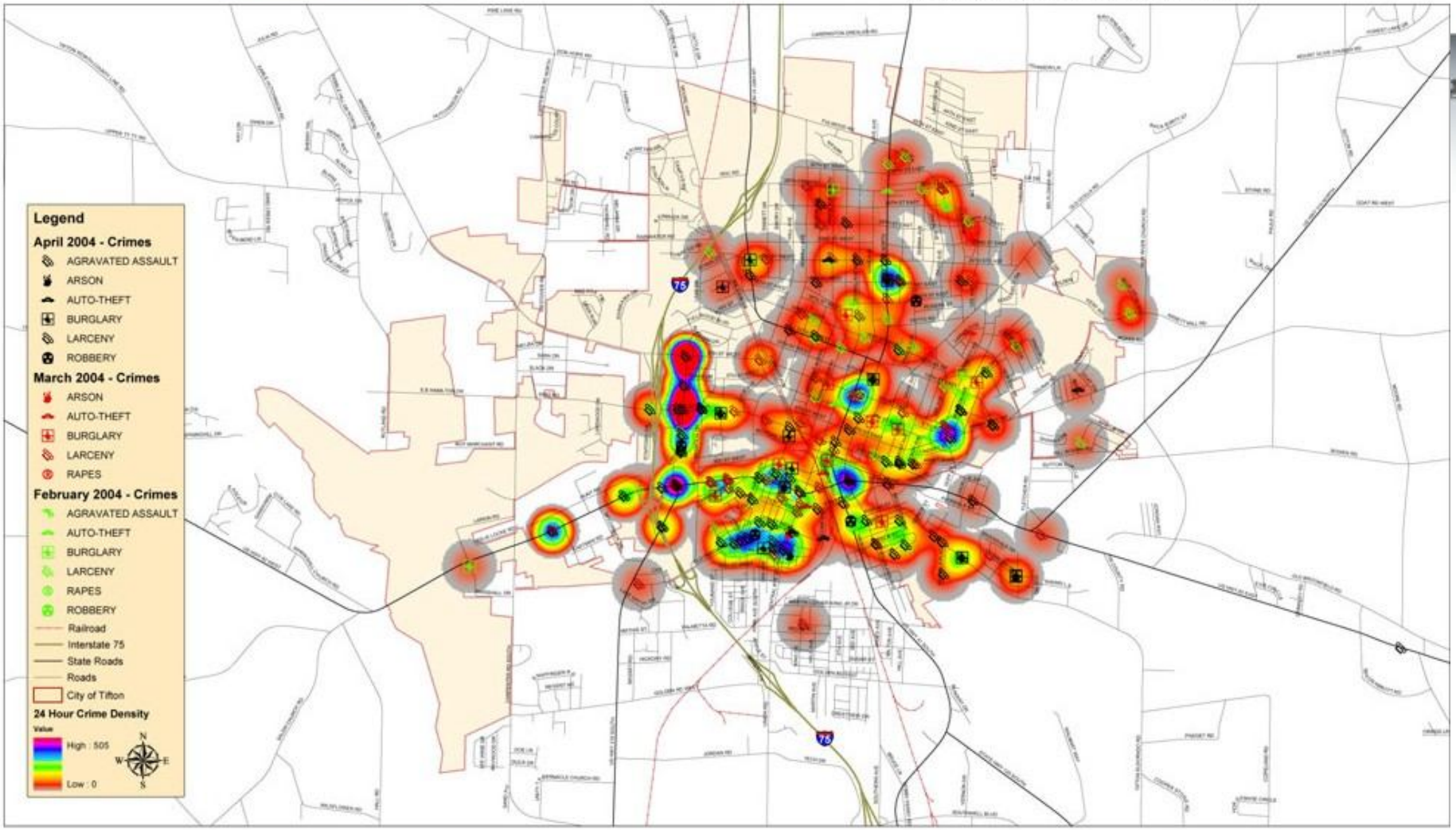
Total Number of Crimes = 127

0 3,200 6,400 12,800 Feet

24 Hour Crime Density for 3 Month Period (below)

Total Number of Crimes = 375

Note:
Density was calculated using a 1000' search radius with a 50' cell size.



What is GIS

- 1. GIS: A type of software
  - A computer system that allows us to handle information about the location of features or phenomena on the Earth's surface
  - Has all the functionality of a conventional DBMS plus much of the functionality of a computer mapping system
  - GIS as a DBMS that allows us to explicitly handle the spatial
  - Common examples:
    - ArcView
    - ArcGIS
    - MapInfo

Approach to GIS

- 3. Approach:
  - Explore the database:
    - In conventional ways
    - AND geographically
  - Allows us to think about the implications of location
  - Allows us to think holistically
  - Should not be restricted by vendor-provided functionality
  - Should be used imaginatively taking into account :
    - the advantages and limitations of geographical information
    - the traditions of humanities scholarship

Geographical Information systems

- 2. GIS: A tool-kit
  - Manipulate spatially:
    - Calculate distances and adjacencies
    - Change projections and scales
    - Integrate disparate sources
  - Analyse spatially:
    - Quantitative analysis
    - Exploratory spatial data analysis
    - Qualitative analysis
  - Visualise data:
    - Maps!
    - Tables, graphs, etc.
    - Animations
    - Virtual landscapes

Geographical Information systems

- Deals with making appropriate or best use of geographical information
- Closely related to GIS but is not application specific
- Examples
  - Analysis techniques
  - Visualisation techniques
  - Algorithms for geographical data

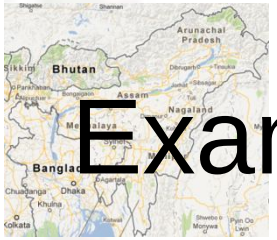
Types of GIS data

- Two types of data are stored for each item in the database

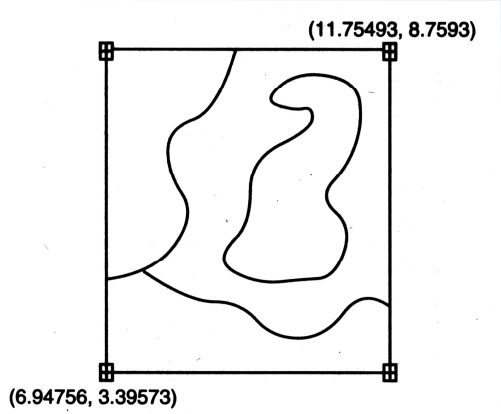
- 1. Attribute data:
  - Says ***what*** a feature is
    - Eg. statistics, text, images, sound, etc.
- 2. Spatial data:
  - Says ***where*** the feature is
  - Co-ordinate based
  - Vector data – discrete features:
    - Points
    - Lines
    - Polygons (zones or areas)
  - Raster data:
    - A continuous surface

Geo referencing Data

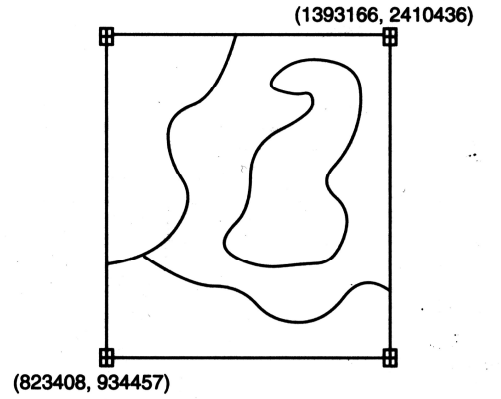
- Capturing data
  - Scanning: all of map converted into raster data
  - Digitising: individual features selected from map as points, lines or polygons
- Geo-referencing
  - Initial scanning digitising gives co-ordinates in inches from bottom left corner of digitiser/scanner
  - Real-world co-ordinates are found for four registration points on the captured data
  - These are used to convert the entire map onto a real-world co-ordinate system



Example of geo-referencing



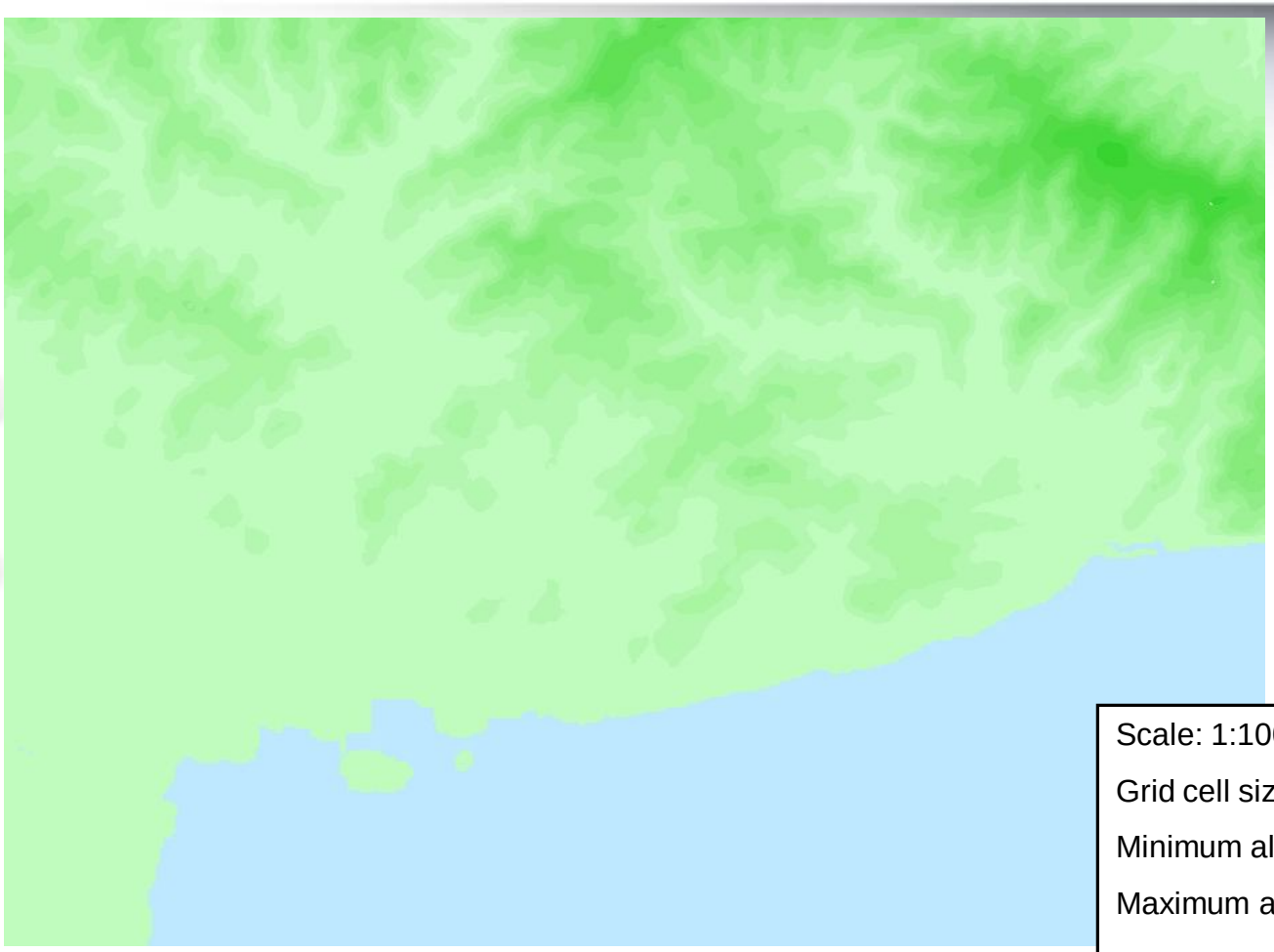
After TRANSFORM
(coverage in real-world coordinates)



Layers

- Data on different themes are stored in separate “layers”
- As each layer is geo-referenced layers from different sources can easily be integrated using location
- This can be used to build up complex models of the real world from widely disparate sources

Raster Example



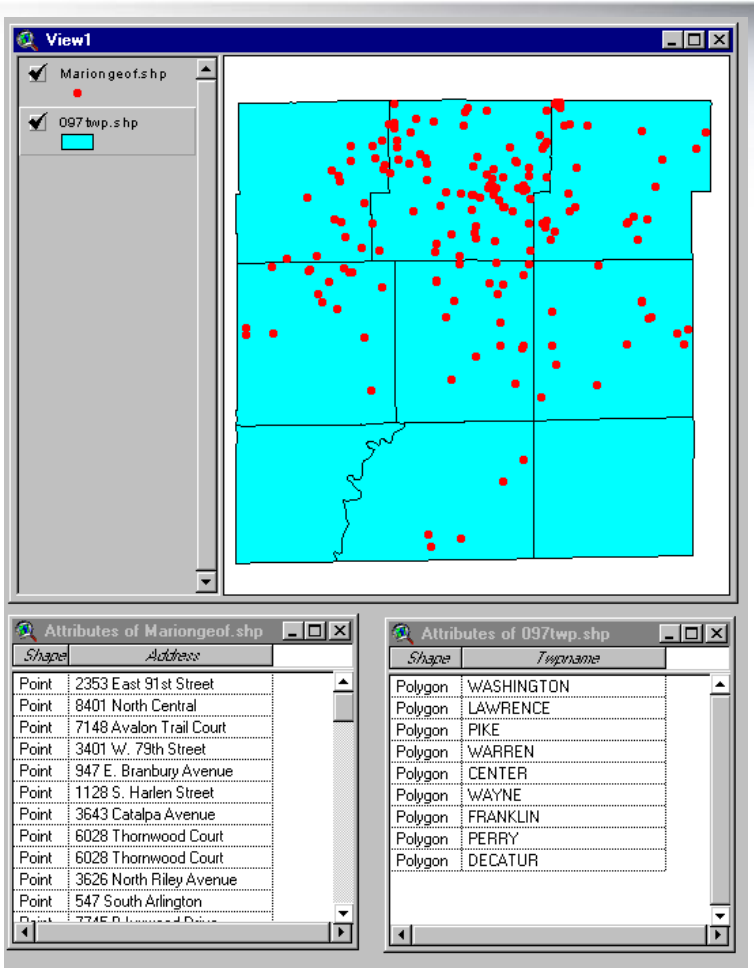
Scale: 1:100,000

Grid cell size: 50 m.

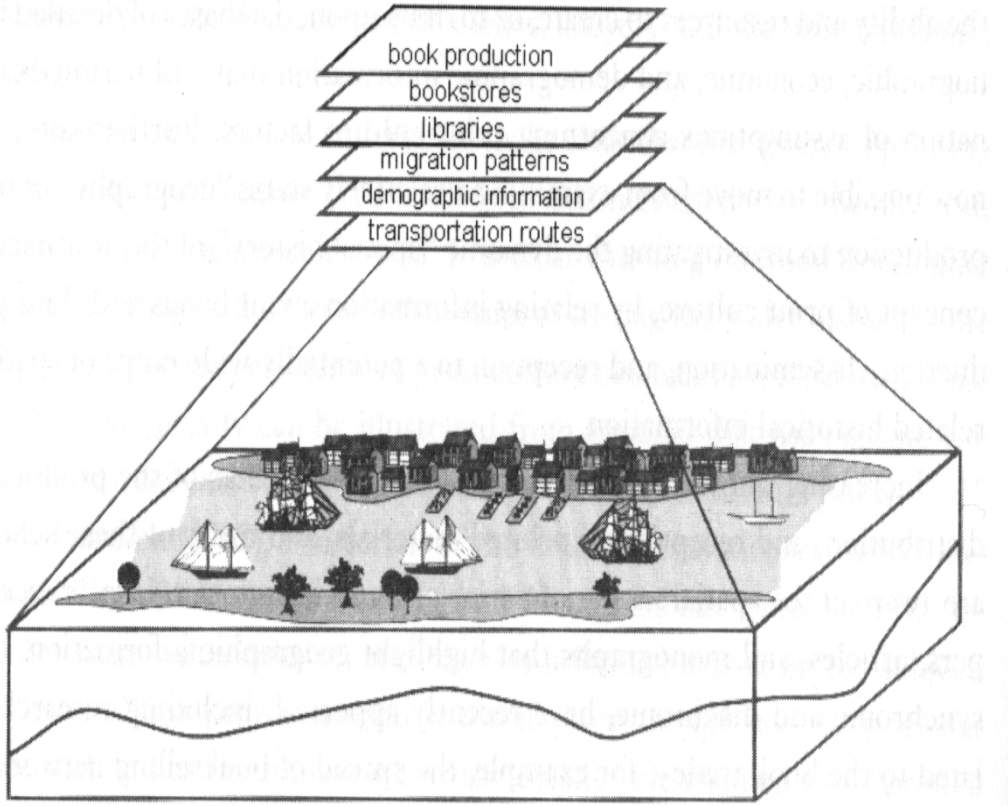
Minimum altitude: 0 m.

Maximum altitude: 174 m.

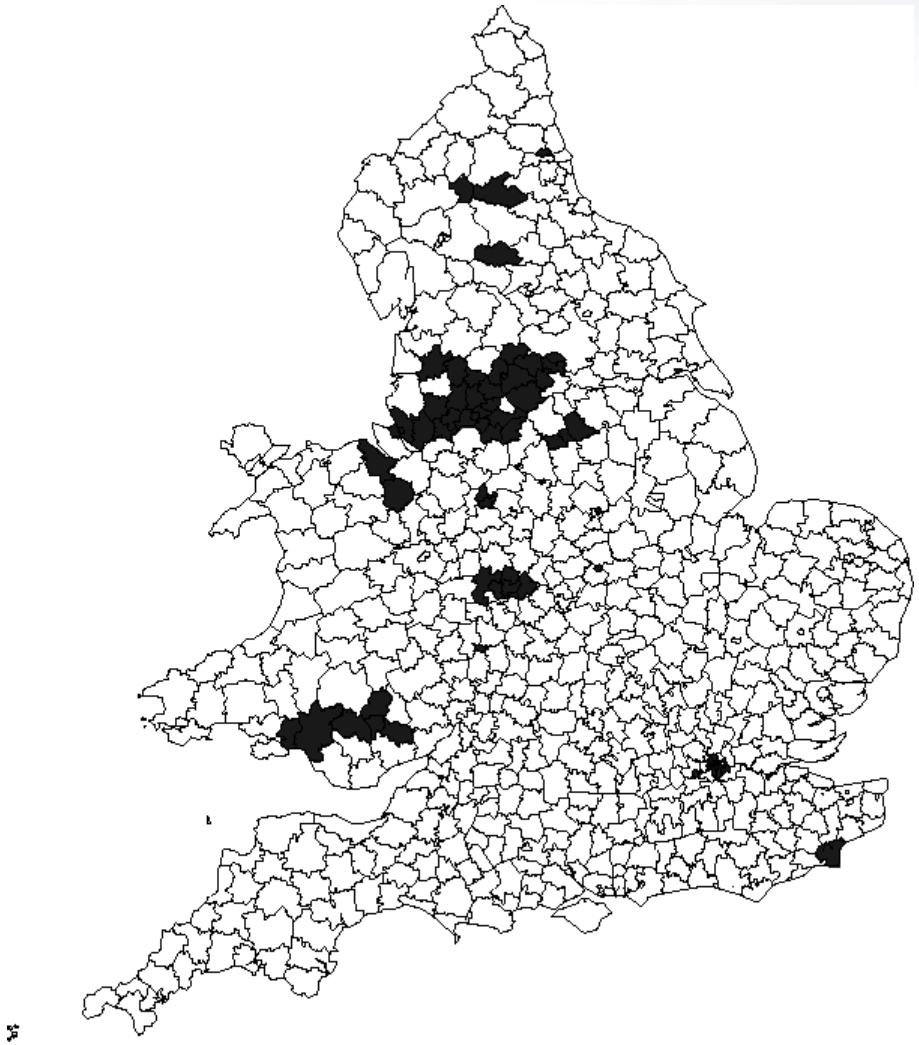
Vector Example



Layering in GIS and Book History



Attribute query: Lung disease in the 1860s



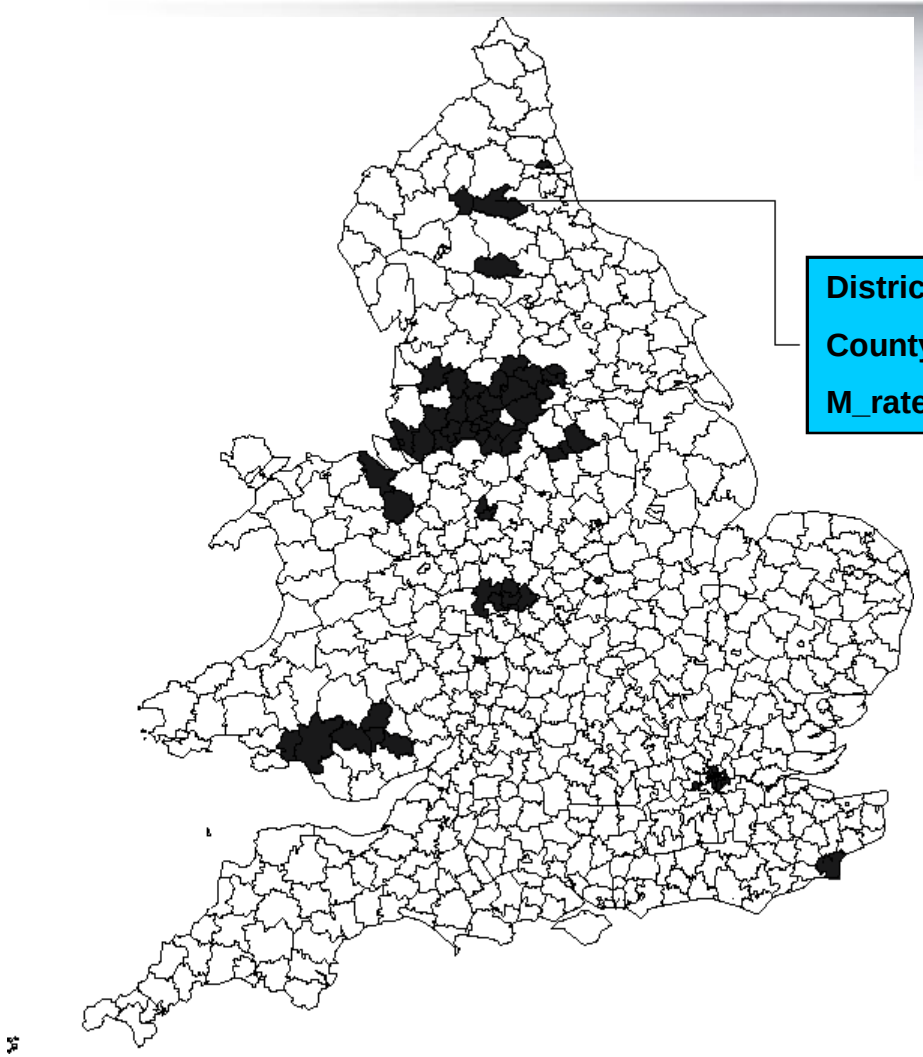
Spatial data: Registration Districts, 1/1/1870

Attribute data: Mortality rate per 1,000 from lung disease among men aged 45-64

Source: Registrar General's Decennial Supplement, 1871

Query: Select areas where mortality rate > 58.0

Spatial query: Lung disease in the 1860s

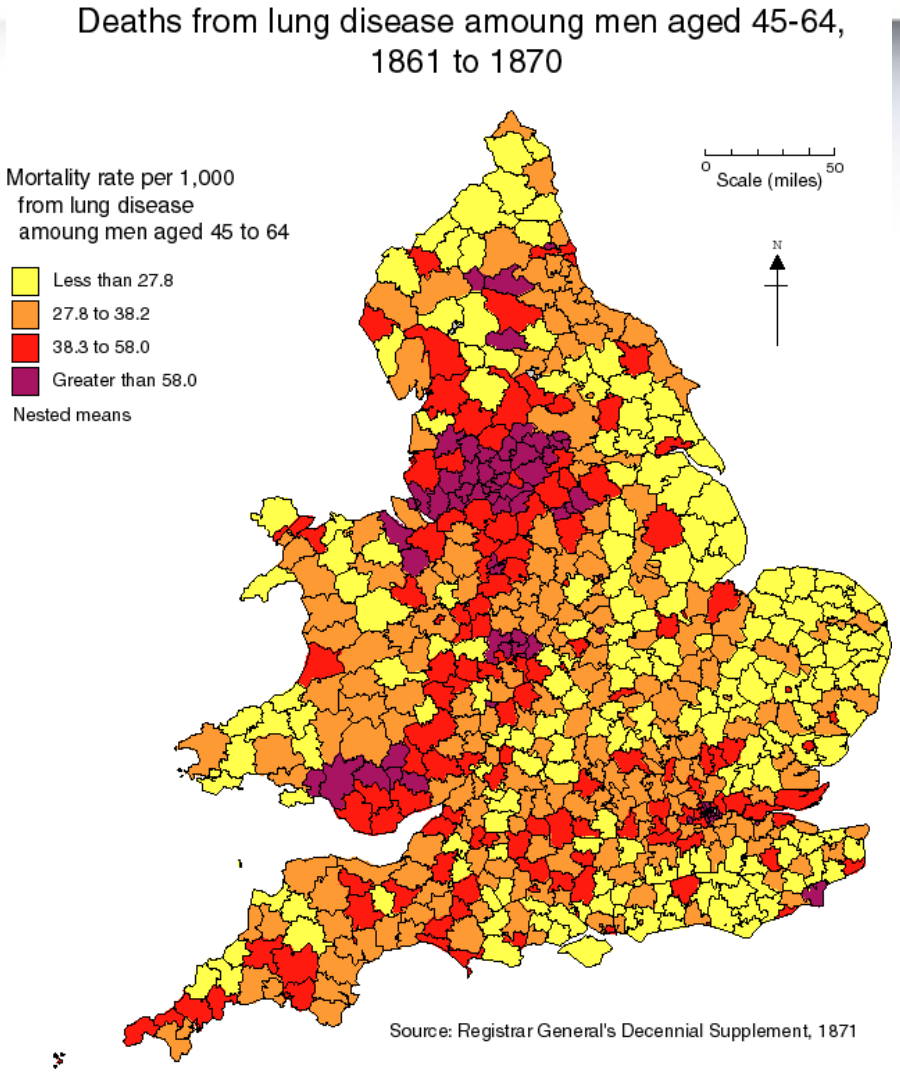


District: Alston with Garrigill

County: Cumberland

M_rate: 68.4

Mapping through attribute query

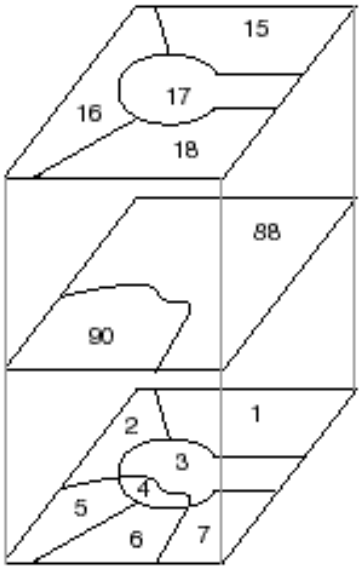


Data integration: Overlay

Joins two layers to create a new layer

The output layer will contain both the spatial AND attribute data from both of the input layers

Spatial Data



Attribute Data

Barony

BARONY-ID	NAME
15	Antrim
16	Glenarm
17	Belfast
18	Toome

Water

WATER-ID	WATER
88	LAND
90	WATER

Output

OUTPUT-ID	BARONY-ID	NAME	WATER-ID	WATER
1	15	Antrim	88	LAND
2	16	Glenarm	88	LAND
3	17	Belfast	88	LAND
4	17	Belfast	90	WATER
5	16	Glenarm	90	WATER
6	18	Toome	90	WATER
7	18	Toome	88	LAND

Querying GIS data

- Attribute query
  - Select features using attribute data (e.g. using SQL)
  - Results can be mapped or presented in conventional database form
  - Can be used to produce maps of subsets of the data or choropleth maps
- Spatial query
  - Clicking on features on the map to find out their attribute values
- Used in combination these are a powerful way of exploring spatial patterns in your data

Conclusions

- Advantages of GIS
  - Exploring both geographical and thematic components of data in a holistic way
  - Stresses geographical aspects of a research question
  - Allows handling and exploration of large volumes of data
  - Allows integration of data from widely disparate sources
  - Allows analysis of data to explicitly incorporate location
  - Allows a wide variety of forms of visualisation
- Limitations of GIS
  - Data are expensive
  - Learning curve on GIS software can be long
  - Shows spatial relationships but does not provide absolute solutions
  - Origins in the Earth sciences and computer science. Solutions may not be appropriate for humanities research

Commonly Used GIS Programs

- Atlas GIS (ESRI)
- ArcView GIS (ESRI)
- ArcInfo GIS (ESRI)
- MapInfo (MapInfo)

Post-mortem examination as an aid in disease diagnosis under field condition

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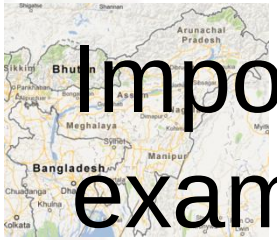
What is post-mortem examination?

- Examination of dead body by systemic dissection to ascertain the cause of death is called post-mortem examination
- In case of animals, it is called necropsy and in human it is called autopsy
- The post-mortem examination should be as thorough as possible
- This should invariably be followed by microbiological, parasitological and histopathological examinations

Eligilibility

- Only registered Veterinary practitioners are eligible to perform the post-mortem examination and write the post-mortem report
- Post-mortem examination is a part of their duty and they have to perform it when needed



Importance of post-mortem examination

- There are several occasions when post-mortem examinations become necessary
- These are :

Outbreak of diseases

Insured animals

Govt. animals

Experimental animals

Veterolegal cases

Types of Post-mortem examination

A
- Complete post-mortem examination

B
- Incomplete post-mortem examination

C
- Cosmetic post-mortem examination

Precaution

- Before proceeding to carry out post-mortem examination, it is absolutely necessary to obtain a written permission from the owner
- In case of Veterolegal cases, permission of local police is a must
- The affected person has to file ‘First information report’ (FIR) before local police
- The police in turn, request the Veterinarian to conduct the post-mortem
- Only then the examination is done

- Post-mortem examination should always be done as soon as possible after death to avoid putrefaction
- Examination is to be avoided in artificial light and should be done in day time
- Accurate change of colour in tissues is not visible in artificial light
- Post-mortem examination is prohibited in case of Anthrax infected animals
- Try to obtain clinical history(anamnesis) which include symptoms and treatment done during life

- When there is no post-mortem room and post-mortem examination is to be done in the village, then it should preferably be done in Govt. land to avoid future litigation
- It is necessary to wear gloves, aprons and gum-boots to avoid contact from zoonotic diseases
- Pathological abnormalities of different organs should be noted during the time of examination
- Post-mortem report should be written immediately after completion of examination, otherwise many important changes are forgotten

Determination of time of death

- It is important on the part of the necropsist to know the time of death
- Generally, it can be obtained from the owner or from the clinical history
- Determination of time and date of death is a difficult task
- Indications as to when death occurred are obtained from the presence or absence of rigor-mortis, abdominal tympany, protrusion of rectum and an odour of putrefaction of dead body

- It is very essential to examine the carcass very carefully for post-mortem changes
- Post-mortem changes are those changes which occur in the body after death
- If the dead body is very fresh and not showing any Post-mortem changes and the blood clot is also of recent origin, it indicates that death has taken place 2 to 4 hours earlier from the time of examination
- If there are Post-mortem changes in the body, then the time of death is determined on the basis of the presence of rigor mortis and on status of the dead body

- Rigor mortis is the shortening and contraction of muscles which occurs after death
- In rigor mortis, body muscles become very hard, stiff and immobilize
- Hardening and stiffness of muscles arise from coagulation of myosin of muscles by lactic acid produced from muscle glycogen due to lack of oxygen
- Presence or absence of rigor-mortis helps in determining the time of death because rigor-mortis appears gradually in the body and also disappears in the same order

- Rigor-mortis first appears in the anterior portion of the dead body and progresses in the posterior direction
- It usually appears first in head, then neck, then fore limbs, then trunk and finally in the hind limbs
- It disappears in the same order
- It disappears first from head and then neck, then from fore legs, then trunk and finally from hind limbs
- Whole body again becomes loose

- Rigor mortis appears only once in the dead body
- Appearance and disappearance of rigor-mortis take certain time after death and this time interval of appearance and disappearance help in determining the time of death
- These vary due to high and low temperature of the atmosphere
- Appearance of rigor-mortis causes stiffness of the whole body in which mouth cannot be opened and joints of legs cannot be twisted or flexed

- In winter, rigor-mortis appears in head within 2-8 hrs after death but in summer, it appears in the head in half to three hours after death
- Rigor –mortis appears in head, neck and fore limb in about 12 hrs and in the whole body in about 15 hrs after death
- If rigor-mortis is present in the whole body during necropsy, indicates that death has taken place between 15-20 hrs earlier from the time of post-mortem examination
- After 20 hrs of death, hind limbs are only found stiff and other parts are loose

- Disappearance of rigor-mortis from whole body takes place in 24-30 hrs and whole body becomes loose and putrid or obnoxious smell is found in the dead body (decomposition or putrefaction)
- Decomposition of tissue occurs by two main types of enzymes
- A) decomposition occurs by own cellular enzymes – autolysis
- B) decomposition occurs by bacterial enzymes - putrefaction

- When the carcass is completely putrified , it means death has occurred more than 30 hrs earlier from the time of post-mortem examination
- While determining the time of death, atmospheric temperature and seasons are taken into account because the appearance of rigor-mortis and putrefaction are hastened by –
- High external temperature
- Violent exercise (racing, fighting or struggling)
- Violent muscular contraction (death due to tetanus or strychnine poisoning)

- Rigor-mortis is retarded or appear very slowly in :
  - low external temperature (winter)
  - emaciated animal
- Amongst the domestic animals sheep putrefy more quickly as the fleece keeps the heat in the body
- Fat animals retain heat owing to poor heat-conduction property of adipose tissue

Report of post-mortem examination

P.M. No…… Date ……

1. Species…… Colour…… Sex ……

2. By whom sent for examination and reason if any ……

3. Date and hour of (A) Death……

(B) P.M.examination…….

4. History ……

5. External appearance and on removal of skin (describe contusions, wounds etc.)…….

6. Mouth and pharynx……..

7. Nasal cavities……..

8. Larynx and trachea……..

9. Oesophagus……..

10. Pleural cavity and lungs……..

11. Pericardium and heart……..

12. Liver and gall bladder……..

13. Spleen……..

14. Stomach and small intestine……..
15. Urinary organs……..
16. Genital organs……..
17. Brain and spinal cord……..
18. Lymph glands in general……
19. Diagnosis (tentative) on the basis of above examination……..
20. Remarks (state if viscera and tissues are sent for chemical, microbiological, histopathological and parasitological examination)…….

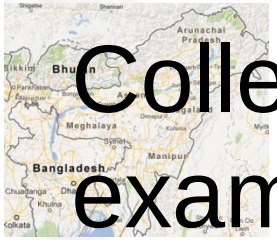
21. Results of microscopical examination and blood etc……

22. Place where P.M.examination was made……

Date…….

Signature of necropsist

Designation with seal



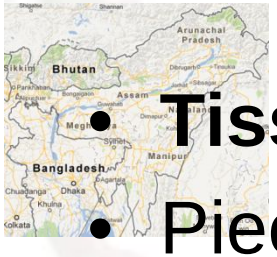
Collection of materials for laboratory examination

- Materials collected at post-mortem should be sent to the laboratory for examination as soon as possible
- Suitable preservative may be used while transporting the materials to the laboratory
- Different preservatives are required for different tests

- Commonly used preservatives in different tests are :
- 1. For histopathological examination – 10% formal saline
- 2. For virological examination – 50% glycerine saline solution
- 3. For preservation of parasites – 70% alcohol
- 4. For bacteriological examination – no preservative in ice box
- 5. For chemical examination of poisons – saturated solution of sodium chloride

Collection of materials in different diseases of animals and birds

- **Impression smears :**
- During post-mortem examination, impression smears of different organs like heart, lungs, spleen, lymph nodes, liver, intestine etc. are prepared
- If any organ is showing lesion, it is necessary to prepare the impression smears of slides from that lesion
- The slides after drying is wrapped in clean paper and sent to the nearest laboratory for examination and report



- **Tissue or organ collection :**
- Pieces of tissue or organ showing lesions are collected during post-mortem in different preservatives as per need
- The collected samples in specimen bottle are sent to the laboratory for examination
- **Collection of materials in different diseases:**

1. Johnes disease:

Lesions : Brain-like corrugation in large intestine

- Materials : Make smear from the thickened areas of intestine, particularly from ileo-caecal valve

- A small portion of intestine showing gross lesions with mesenteric lymph nodes for cultural and histopathological examination

2. Tuberculosis:

- Lesions : Nodular lesions of different size in different organs
- Materials : Impression smears from lesions and lymph glands (mediastinal or mesenteric)
- Tissue pieces from lesions for cultural and histopathological examination

3. Black quarter(B.Q.)

Lesions : Gangrenous myositis in heavy muscle

- Materials : Smears from affected muscle
- Pieces of affected muscle for cultural and histopathological examination

- **4. Enterotoxaemia :**
- Lesions : Congestion and haemorrhage in abomasum and intestine
- Hydropericardium, epicardial and endocardial haemorrhage(petechiae) in heart
- Kidney – soft and pulpy
- Materials : Loop of intestine

5.Anthrax

- Materials : Cut a portion of the ear to prepare blood smear
- Take a portion of the skin in sterile glass container for Ascolis test

6. Haemorrhagic septicaemia(H.S.)

- Lesions : Petechial haemorrhage in various organs, oedematous swelling in throat region and pneumonia
- Materials : Smears from heart, lung, liver and submaxillary swelling
- Pieces of lung, liver, spleen, lymph nodes, heart and intestine for cultural and histopathololological examination

7. Strangles :

Lesions : Suppuration in upper respiratory tract and abscess formation in sub-maxillary and pharyngeal lymph nodes

- Materials : Smears from pus of affected part
- Pieces of affected lymph nodes, lungs, spleen and trachea for cultural and histopathololological examination

8. Glanders :

- Lesions : Ulcers in nasal cavity, tubercle-like lesion in lung, cutaneous ulcers along tortuous thick-walled lymphatics. Abscess in superficial lymph nodes
- Materials : Pieces of lung and superficial lymph node for cultural and histopathololological examination and for strauss test

9. Swine Erysipelas :

- Lesions : Septicaemia, vegetative endocarditis, arthritis and diamond shaped red patches (erythema) on the skin
- Materials : Portion of affected organs like liver, pieces of affected valve and skin

10. Leptospirosis :

- Lesions: Haemorrhage in lungs, stomach, intestine, liver, kidneys, heart and bladder and changes of hepatitis and nephritis
- Materials : Portions of liver, kidney and spleen

11. Actinomycosis :

- Lesions : Hard, suppurative, irregular swelling on mandible or maxilla
- Materials : Smears from pus,
- Portion of lesions of mandible and maxilla

12. Actinobacillosis :

- Lesions : Hard swelling on tongue, lymph nodes of head, neck, thorax and rarely in lungs
- Materials : Smears from affected area
- Portions of hard swelling

13. Brucellosis :

- Lesions : Aborted fetus, morocco leather-like appearance of placenta with necrosis
- Materials : stomach content from aborted fetus in sterile tube
- Pieces of stomach, liver and placenta

14. Rabies :

- Lesion : Brain may not show any lesion except congestion
- Materials : Impression smears from brain
- Pieces of brain tissue from hippocampus (canines) or cerebellum (herbivores)

15. Foot and Mouth Disease :

- Lesions : Vesicles and erosions on lips, dorsum of tongue and palate and on the skin near coronary band in inter-digital space
- Materials : Epithelial covering of vesicular lesions

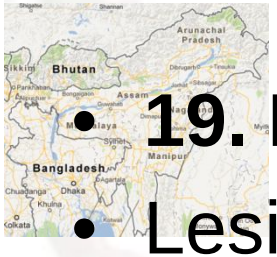
16. Rinderpest :

- Lesions : Lymph nodes are enlarged. Focal areas of shallow erosions inside the lower lip, adjacent to gum, ventral free portion of tongue, oesophagus and abomasum
- Petechial haemorrhage in large intestine (zebra markings)
- Materials : Portions of large intestine, pieces of spleen and lymph glands

- **17. PPR (Pestes-des-petits) :**
- Lesions : Necrotic areas on the mucosa of nostrils and turbinates, hydrothorax, consolidation of lungs(antero-ventral part), white necrotic debris in oral lesions, erosive lesions in GIT, streaks of haemorrhage on mucosal crest of caeco-colic junction of large intestine. Mesenteric lymph nodes – enlarged, congested and oedematous
- Materials : Lung, liver, spleen, lymph node and erosive lesions

18. Classical Swine Fever :

- Lesions : Petechial haemorrhage beneath the kidney capsule(turkey egg appearance), infarction in spleen, haemorrhage and necrosis in tonsil and lymph nodes, button ulcer in large intestine and congestion of brain
- Materials : Pieces of affected organs including lymphoid tissues and brain



- **19. Porcine Circovirus infection :**
- Lesions : Affects weaned piglets, emaciated, rough hair coat. Mucous membrane – pale and icteric. Enlarged peripheral lymph nodes
- Materials : Pieces of lymph nodes and spleen
- **20. Porcine Respiratory and Reproductive Syndrome(PRRS)**
- Lesions : Severe pneumonia in neonatal piglets, premature furrowing, still born, autolysed and mummified foetus, brain congested
- Materials : pieces of reproductive tract, lungs and lymph nodes(mediastinal)

21. Canine distemper :

- Lesions : Coryza, pneumonia, cojunctivitis, vesiculo-pustular lesions on skin and hyperkeratosis of foot pad
- Materials : Portion of lungs and mediastinal lymph nodes, skin, kidneys, liver and trachea

22. Infectious Canine Hepatitis :

- Lesions : Enlargement of liver, spleen and lymph nodes , haemorrhage in many organs and oedematous thickening of gall bladder mucosa
- Materials : Portions of liver, gall bladder, kidney, spleen and lymph nodes

23. Theileriasis :

- Lesions : Enlargement of lymph nodes, pulmonary oedema and emphysema, petechal haemorrhages and punched out ulcers on the mucosa of abomasum
- Materials : Smears from lymph nodes, spleen, liver and portions of lymph nodes, spleen and abomasum

24. Babesiosis :

- Lesions : Enlargement of spleen, liver, gastro-enteritis, icterus, lungs-oedematous and red coloured urine in bladder
- Materials : Smears from heart, spleen, kidneys and portions of affected organs

25. Avian influenza :

- Lesions : Swelling of head and face, haemorrhage below the skin, feet with cyanosis of head and wattle
- Various congestive and haemorrhagic lesions on the skin, liver, spleen , heart, kidneys and lungs
- Materials : Faeces or intestinal contents and trachea

26. Ranikhet Disease (New castle Disease) :

- Lesions : haemorrhage in proventriculus, caecal tonsil, necrotic and haemorrhagic lesions in intestine, pneumonia and congestion of brain
- Materials : Bone marrow, portions of lung, trachea, brain, liver, spleen and other affected tissues

27. Infectious Bursal Disease (Gum-Boro Disease)

- Lesions : Enlarged, oedematous and haemorrhagic bursa, swollen kidneys & spleen, haemorrhage in thigh and breast muscles and lining mucosa of proventriculus
- Materials : pieces of bursa, spleen, kidney and liver

28. Avian encephalomyelitis :

- Lesions : Inflammation of brain and spinal cord and whitish area in the muscularis of proventriculus
- Materials : Portions of brain, spinal cord, pancreas and proventriculus

29. Marek's Disease :

- Lesions : Affected nerves are up to 2-3 times more thicker than normal with loss of striation and glistening appearance (classical form)
- Enlargement and tumour formation in liver, spleen, kidneys, lungs, ovary, testes, proventriculus and heart (acute form)
- Materials : Pieces of affected tissue

30. Lymphoid Leucosis :

Lesions: Liver is greatly enlarged with enlargement of spleen, bursa of Fabricius, kidneys and ovary. Tumour almost always involve liver, spleen and bursa

Lesions : Vaginal and cloacal swab, pieces of tissue containing tumour

31. Fowl pox :

- Lesions : Presence of scabs or pox lesion on eyelids, comb, wattle, ear and nose
- Materials : Dead bird or scabs

32. Egg Drop Syndrome:

- Lesions: Inactive ovaries, decrease in size of oviducts(atrophy)
- Thin-shelled, soft-shelled and shell-less eggs
- Materials: Pieces of ovary and oviducts

33. Chicken Infectious Anaemia:

- Lesions: Marked reduction in the size of thymus, bursa of Fabricius and spleen
- Bone marrow changes from a red colour to a yellow or white colour
- Swollen liver and haemorrhage in proventriculus, under the skin and muscles
- Materials: Bone marrow, thymus, bursa and spleen

34. Inclusion Body Hepatitis:

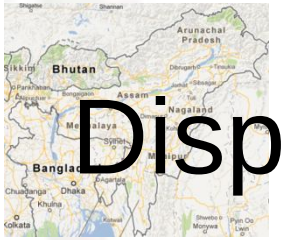
- Lesions: pale, friable and swollen liver
- Haemorrhage in the liver and skeletal muscles, kidneys are swollen
- Materials: Pieces of liver and kidneys

35. Hydropericardium-hepatitis syndrome(Leechi disease, Angara disease)

- Lesions: Upto 10ml of clear fluid in the pericardial sac, pulmonary oedema, liver and kidneys enlarged, pale and friable
- Materials: Pericardial fluid, pieces of liver and kidneys

- **36. Chronic Respiratory Disease:**
- Lesions : Inflammatory exudate in nasal passage, trachea, bronchi and airsacs
- Airsacs usually contain cheese-like inflammatory material
- Pericarditis, perihepatitis and pneumonia
- Materials : Swabs from nasal cavity, trachea, airsacs and lungs
- **37. Coccidiosis :**
- Lesions : Haemorrhage in caecum and intestine
- Materials : Pieces of affected caecum and intestine

- **38. Aspergillosis :**
- Lesions : Nodular growth on lungs or diffuse pneumonia and thickening of wall of air sacs with white moldy growth on the surface
- Materials : pieces of lungs and air sac



Dispatch of materials

- While dispatching materials to laboratory for any kind of test, the materials should be kept in preservatives in a sealed container.
- A letter containing the following information should be attached:
  1. Species, breed, age, sex of the livestock/bird
  2. Source and date of collection
  3. History of the disease with symptoms

4. Duration of outbreak/disease
5. Approximate number of animals in farm/locality
6. Approximate number of animals affected
7. Number of animals died
8. Nature of feed (specify if change is made)
9. Post mortem changes recorded
10. Time between death and collection of material
11. Type of material despatched (with no. of tissues of each organ if H.P)

- 12. Type of preservative used, if any
- 13. Nature of test(s) required
- 14. Your tentative diagnosis
- A duplicate letter should always be sent by post.

Chemical examination of cases of poison

- For determination of poison, it is necessary to collect the following materials:

(i) Pieces of liver, kidney, intestine, lungs and stomach

(ii) Loops of intestine and stomach with content

Preservative:

1. Saturated solution of sodium chloride or common salt
2. 90% alcohol (ethyl)

(iii) Suspected plant with leaves/grass /hay/silage or concentrate feed without any preservative in a sealed packet/container.

- These materials can be sent directly to the Director, Forensic Science Laboratory or through Disease Investigation Officer of the State
- The collected materials should be properly placed in a container with the preservative, sealed and dispatched, preferably through a special messenger
- A letter describing the symptoms, the materials with preservative, your suspicion as well as the type of poison(if possible) to be tested for, should accompany the material
- A duplicate letter should always be sent by post

Record Register in Laboratory

Date	Sample No.	Species of animals	History	Specimen	Preservatives	Lab result/diagnosis

Thank You

ADVANCED ANIMAL DISEASE DIAGNOSIS AND DISEASE MANAGEMENT CENTRES

- **TOPIC: Group activity: Design of Pathology laboratory and creation of facilities**
- **Dr. D.C. Pathak and Dr. T.N. Upadhyaya**

Department of pathology

College of Veterinary Science

AAU, Khanapara, Guwahati-781022

Requirements for Histopathology Laboratory.

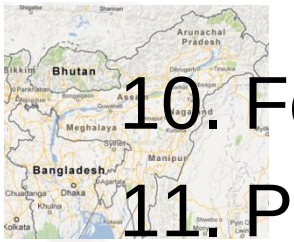
- **A. Instruments:**

1. Microtome machine: Rotary/ Automatic/ Semiautomatic
2. Cryostat machine
3. Tissue floatation bath

4. Microscopes: Olympus, Magnus etc.
5. Hot air oven
6. Automatic tissue processor
7. Paraffin embedding bath
8. Automatic Knife sharpener
9. Water distillation apperatus

- **B. Appliances:**

1. Microtome blade: reusable, disposable
2. Microslides
3. Coverslips
4. Funnels
5. Glass bottles fo keeping the stains
6. Staining jars
7. Tissue capsules
8. L- moulds
9. Block holders



10. Forceps
11. Paint brush (small)
12. Blade (Shaving blade/ BP knife with holder)
13. Knife holder for knife sharpening
14. Honing stone for knife sharpening
15. Spirit lamp
16. Electric heater
17. Glass beakers
18. Measuring cylinders
19. Slide tray

- **C. Chemicals:**

1. Absolute alcohol
2. 90% alcohol
3. Rectified Spirit
4. Xylol/Xylene
5. Paraffin wax
6. DPX
7. Egg albumin
8. HCl for acid alcohol
9. Ammonia solution

10. Haematoxylin powder
11. Eosin powder
12. Alum
13. Formaldehyde
14. Thymol

- **D) Furniture:**

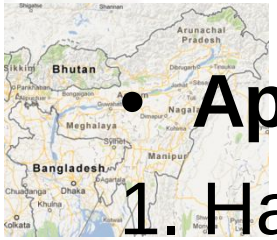
1. Laboratory table
2. Washing sink
3. water source with overhead water tank
4. Table-chair
5. Almirah/Rack
6. Stools

B. CLINICAL PATHOLOGY LABORATORY

A. Instruments:

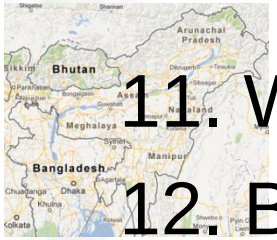
1. PCR Facility
2. Biochemical auto analyzer
3. Haematological auto analyzer
4. UV /Vis Spectrophotometer
5. ELISA reader
6. pH meter
7. Water Distillation set
8. Centrifuge machine
9. Microhaematocrit machine

10. Hot air oven
11. Autoclave
12. Refrigerator
13. Urinometer
14. Microscope
15. Deep freeze

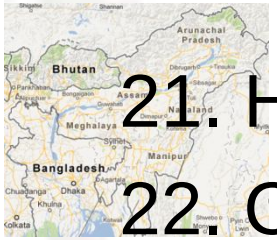


- **Appliances:**

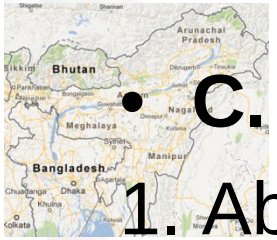
1. Haemocytometer
2. Haemometer/ Haemoglobinometer
3. Pipette with stand
4. Micropipettes with stand
5. Test tubes with stand
6. Test tube holder
7. Rubber teats
8. Spirit lamps
9. Glass slides
10. Coverslips



11. Watch glass
12. Blood collecting vial/Vacu-container
13. Serum vials
14. Absorbant cotton
15. Non-absorbant cotton
16. Filter paper
17. Funnels
18. Beaker
19. Flasks
20. Measuring cylinder



21. Haematocrit tube (Wintrobe)
22. Capillary tube for Microhaematocrit machine
23. Disposable syringe
24. Microslide and cover slips
25. Centrifuge tubes
26. Wintrobe tubes for ESR & PCV
27. Pasteur pipette/long nozzled pipette/needle



- **C. Chemicals and reagents**

1. Absolute alcohol
2. Spirit
3. Methanol
4. Wright’s stain
5. Giemsa stain
6. Leishman’s stain
7. Gram’s stain
8. Methylene blue stain
9. Acid fast stain
10. Benedict’s reagent

11. Robert’s reagent
12. Nitric acid
13. Hydrogen peroxide
14. Ammonia
15. Benzidine
16. Nitroprusside
17. RBC diluting fluid
18. WBC diluting fluid
19. N/10 HCl
20. Anticoagulant (EDTA, Heparin etc)

COMPOSITION OF SOME STAINS

- **<u>Haematoxylin (Delafield's)</u>**

Haematoxylin crystals

Ammonium or potassium alum

Glycerine

Alcohol

- **Harris haematoxylin**

2,4,5- mercuric chloride

Glacial acetic acid

- 1% Stock alcoholic eosin

Eosin Y water soluble

Dist. Water

95% alcohol

Glacial acetic acid

- ***Gram's Stain***

  Gram's iodine

  Iodum

  Pot iodide

  Dist. Water

- ***Ziehl Neelsen Stain***

  1. Carbol fuchsin solution

  Phenol crystal (melted)

  Alcohol

  Basic fuchsin

  Dist. Water

- 2. 1% acid alcohol

70% alcohol: 1000 ml

Conc. HCl: 10 ml

- 3. Ammonia water:

Tap water: 1000 ml

28% Ammonium hydroxide: 2-3 ml

- 4. 1% Sulfuric acid solution (Stock)

Conc. Sulfuric acid: 1 ml

Dist. Water : 100 ml

- **Methylene blue solution (Stock)**

Methylene blue: 1.4 g

95% alcohol: 100 ml

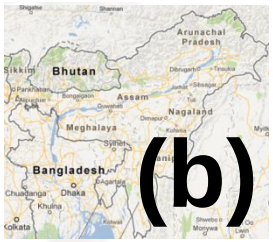
Methylene blue solution (Working)

M.B. Stock: 10 ml

Tap water: 90 ml

Companies dealing with (a) the chemicals:

1. Hi media
2. Fisher Scientific
3. Central Drug House (CDH) Private Limited. 7/28 Vardaan House, Mahavir Street, Ansari Road, Daria Ganj, New Delhi-110002
4. Qualigens Fine Chemicals. Dr. Annie Basant Road, Mumbai-400025
5. MERCK



(b) Instruments/Lab equipments

1. Narang Scientific Works Pvt. Ltd., New Delhi
2. MAC (Marco Scientific Works Pvt. Ltd).; B-35/3, G. T. Karnal Road Industrial Area, Delhi-110033

- **(c) Glass wares/Plastic wares**

1. Borosil
2. Tarsons

- **Authorized Distributors:**

1. North East Chemicals
2. S. B. Suppliers

REFERENCES

- Manual of Histologic Staining Methods of the Armed Forces Institutes of Pathology. 3rd edition, edited by LEE G. LUNA, HT (Ascp). The Blackston Division. Mc Grow Hill Book Company. New York, Toronto, London, Sydney.

Role of the laboratory in investigation of disease outbreak

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Laboratories and Disease Outbreaks

Before the outbreak

- Early warning signals
- Outbreak detection

During the outbreak

- Outbreak response and management

In between outbreaks

- Trend monitoring
- Intervention evaluation
- Monitoring progress towards a control objective

Early warning signals

- **Detection of pathogens that have potential to spread**

- **Sentinel events requiring early control measures**
  - **Isolation of a single epidemic prone isolate (e.g. non-invasive *E. coli* isolated from heart of dead animal)**
  - **Emergence of resistant strains in the community (e.g. multi-drug resistant enteric bacteria)**

Outbreak detection

- Outbreak detection by the laboratory
- Outbreak detection with assistance from the laboratory

Outbreak detection by the lab

- **Identification of a cluster of:**
  - **Infections with an unusual pathogen**
  - **Specific subtype of a pathogen**
    - **–Outbreak of antibiotic-resistant strains**
    - **–Subtypes of a pathogen (e.g. *Shigella dysenteriae* type I)**

Reference centres may capture outbreaks disseminated over a large area

Outbreak detection with lab help

- **Epidemiologist captures an increased incidence**

Laboratory:

- **Confirms the diagnosis**
- **Allows for a more specific case definition**
- **Detects a new pathogen**
- **Provides additional details on the pathogen (e.g., type, subtypes etc.)**

- **Effective participation of the laboratory in surveillance requires good communication between the epidemiologists and the laboratories**

Laboratory role during outbreaks

- Laboratory confirmation of early cases
- Identification of new pathogens
- Typing of the pathogen
- Antimicrobial susceptibility testing to guide treatment
- Post-outbreak surveillance
- Environmental investigations
- Detection of carriers

Laboratory role during outbreaks

For new and emerging pathogens:

- **Identify the pathogen**
- **Develop laboratory tests**
- **Suggest treatment/management**

Monitoring endemic disease trends

- **Confirm diagnosis**
  - **Case definitions that include laboratory criteria**
- **Monitor resistance patterns**
- **Monitor subtypes of a pathogen**

Monitoring endemic disease trends

Examples:

- **Circulating strains of CSFV**
  - **Impact on diagnostic protocols**
  - **Impact on immunization policies**

- **Antibiotic resistance**
  - **Methicilin resistant *Staphylococcus aureus***
  - **Vancomycin resistant *Enterococcus***
  - **Karbapenem resistant *K. pneumoniae***

Eradication/elimination monitoring

- The elimination phase requires more specific tests as positive predictive value decreases

- Laboratory confirmed diagnosis
  - Avian Influenza
  - Swine flu

- Typing helps identifying the origin

Establishing laboratory support for continuous disease surveillance

- Identify diseases of economic importance
- List diseases that require laboratory confirmation
- Determine tests to be performed
- Map laboratory facilities and human resources, including reference laboratories
- Establish laboratory networking
- Identify a focal person to coordinate laboratory activities
- Determine information flow

Contd....

- Define roles and responsibilities, identify referral system
- Ensure supplies, logistics, guidelines and forms
- Organize communication between laboratory and epidemiologist
- Plan quality assurance, biosafety and waste management
- Supervise and monitor
- Develop outbreak preparedness and response plans

Contd....

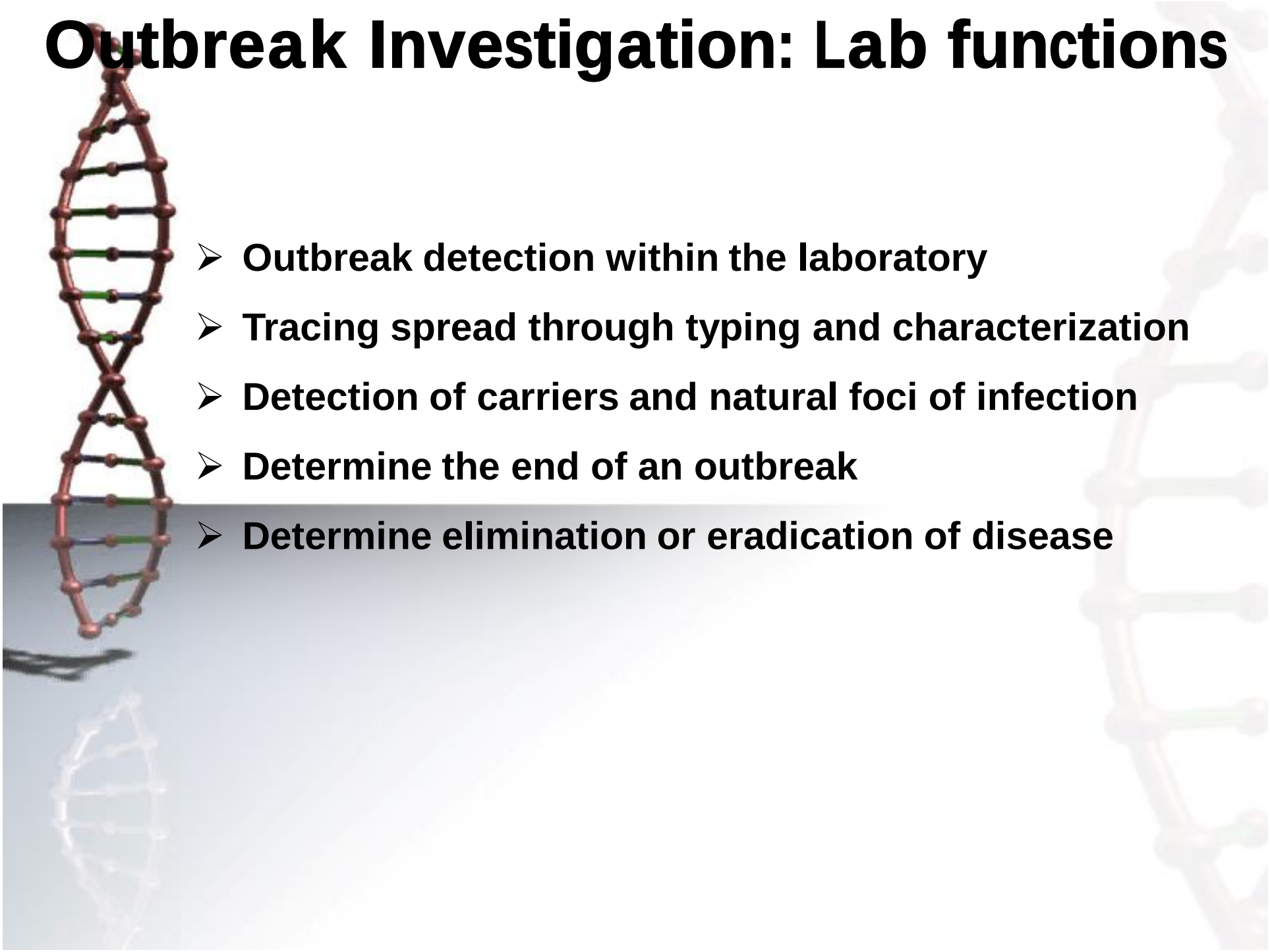
➢Peripheral level objectives

- Diagnosis and early warning signals

➢Routine lab surveillance with intensification before outbreak season

- Environmental monitoring
- Epidemic prone disease monitoring
- Proper collection, transport and storage of samples
- Reporting of results

Outbreak Investigation: Lab functions



- Outbreak detection within the laboratory
- Tracing spread through typing and characterization
- Detection of carriers and natural foci of infection
- Determine the end of an outbreak
- Determine elimination or eradication of disease

But before investigating an outbreak…

PREPARE for an outbreak if it is likely!!

DIAGNOSIS OF PARASITIC DISEASES

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Introduction:

- Parasitic diseases inclusive of Helminths, Arthropods and Protozoans are compulsorily associated with the host/hosts.
- Different stages of these organisms *viz*. Egg/ova/larva/cyst/oocysts/trophozoites are found in the faeces/urine/nasal discharge/sputum/blood according to infection agent.
- Identification of these stages are strongly required to detect and confirm the disease agent.

- Examination of faecal samples is done to detect helminth and some protozoa.
- Infestation of skin with mange mites can be detected by examination of skin scrapings.
- Haemoprotozoan parasites as well as larvae of filarial nematodes can be detected by examination of blood.
- Diagnosis of nasal schistomosis can be done by examination of nasal scrapings.
- Acid Ether Method is useful for diagnosis of visceral Schistosomiasis.

Examination of faecal samples

- Gross examination of faeces:
  - Consistency
  - Colour
  - Blood
  - Mucus
  - Age of the faeces
  - Gross parasite

Microscopic examination:

Simple floatation:

1. To concentrate the parasitic eggs, cysts or oocysts using floatation solution having different specific gravity.
2. Saturated sodium chloride (sp.gr. 1.20) solution for floating Trichostrongylid and Strongylid eggs

3. Saturated sucrose solution (sp. gr. 1.12-1.30) for floating coccidia oocyst.
4. Zinc sulphate solution (33%, sp. gr. 1.18) for detection of Giardia cyst and Fasciola eggs.

A. Centrifugation method:

This method is use to save time and to obtain greater accuracy

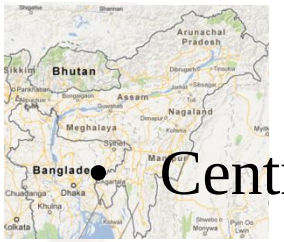
B. **Sedimentation method**

To detect trematode and cestode eggs or protozoa cyst

Formol-Ether technique

To detect Giardia cyst

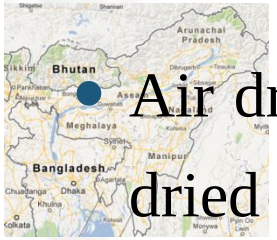
- Emulsify about 1g of faeces in a pestle and mortar with 5ml of 10% formol saline.
- Strain through a strainer into a centrifuge tube.
- Add equal volume of commercial ether, insert a rubber stopper and shake vigorously.
- Remove the stopper and let stand for 2 minutes.



- Centrifuge at 2000rpm for 2 minutes. A ring of faecal debris will appear in between the ether (top) and formalin (middle) leaving the sediment at the bottom.
- Loosen the debris ring with an applicator
- Pour off the supernatant and debris ring carefully without disturbing the sediment.
- Add a drop of physiological saline to the sediment and mix well.
- Take a drop of sediment with a pipette on a clean glass slide, add a drop of iodine solution, put a coverslip and examine under microscope.

Detection of *Cryptosporidium* oocyst

- Sheather's sucrose floatation method can be applied
- The oocysts appeared as round or oval, refractile bodies with a thin cytoplasmic membrane
- The positive sample is then subjected to modified Ziehl-Neelsen staining and Kinyoun's staining methods
  - **Modified Ziehl-Neelsen staining:**
    - With a wooden applicator/stick a thin faecal smear is made on a clean greese-free micro slide and air dried.



- Air dried smear will be fixed in absolute methanol for 5 mins, air dried and then transiently passed over flame.
- Stain the smear with strong carbol fuchsin solution for 20 mins.
- After staining, rinsed thoroughly under running tap water.
- Decolorized with acid alcohol (1%) for 10-15 secs and rinsed thoroughly in tap water.
- Followed by counter staining with Malachite green (5%) for 5 mins.
- Finally, rinsed the slide thoroughly in tap water, air dried and examined microscopically under high power (400X) and oil immersion objective (1000X).

Kinyoun's staining method

- Thin faecal smear is prepared on a clean, grease free glass slide with a wooden applicator/stick and air dried.
- Fixed the slide in absolute methanol for 5-10 mins and stained with Kinyoun's acid fast stain (Garcia and Bruckner, 1993) for 2 mins followed by rinsing thoroughly in tap water.
- Decolorized the slide with 10% sulphuric acid (H_2SO_4) followed by rinsing in tap water.
- Counter stained with methylene blue (0.3%) for 1 min, rinsed in tap water, air dried and examined microscopically under high power (400X) and oil immersion objective (1000X) for detection of Cryptosporidium oocysts against blue background.

Examination of nasal scrapping

- Nasal scrapping taken with scalpel or with scoop in 10% formalin.
- This material is examined under microscope for detection of the characteristic eggs of *Schistosoma nasale* which is boomerang shaped.

Acid ether method for *Schistosoma indicum*

- One gram of faeces is emulsified with 5ml of 40% HCl,
- Material is filtered through two layers of moist gauze in a centrifuge tube.
- Equal quantity of ether is added and shaked vigorously.
- Centrifuged at 1500 rpm for one minute.
- The debris at the middle layer are loosened with an wooden applicant.
- The supernatant is discarded and the sediment is examined for presence of schistosome eggs.

LARVAL NEMATODE CULTURE:-

- To detect light infection with hookworm, strongyloides or Trichostrongylus.

Method for recovery:-

- Harada Mori tube and petridish culture method.
- Baermann technique.

Larval nematode culture
HARADA-MORI filter paper strip culture:

To detect light infection with hookworm, *Strongyloides stercoralis* and *Trichostrongylus* sp. and to facilitate specific identification

- Approximately 0.5g of faeces is smeared on a narrow sheet of filter paper (3 x 16cm). About 5cm space on one end and 1cm on the other end are left unsmeared.
- The filter paper is then placed in a test tube 18cm in height and 2cm in diameter, with the unsmeared end (5cm) toward the bottom. 2-3ml of water is introduced into the tube.
- The test tube is covered with a piece of gauze cloth held in place by a rubber band.
- The tube is incubated at 24-28^{0}C for about 7-10 days.
- Eggs of hookworm and certain other nematodes hatch on the filter paper develop into infective larvae, migrate towards the water source.
- The tubes are examined under low power magnification for the presence of larvae. The fluid may be removed by a pipette and larvae identified under higher magnification.

Baermann technique

- The Baermann technique is used to recover the larvae of roundworms from faeces, soil or animal tissues.

Some recent Techniques of faecal examination

- Gomori's trichrome stain: for superior to all other test in the detection of Cryptosporidium oocyst
- Kato-Katz technique, cellophane faecal thick smear: for all helmints (Intestinal Schistosomiosis)
- Auramine –Phenol staining : for Cryptosporidium
- Benedicts and Nimasary method

DIAGONOSIS OF MICROFILARIASIS IN DOGS:-

Modified Knotts Method.

- Mix 2 ml of blood sample with 10ml of 2% freshly prepared formalin solution.
- Gently invert the tube 3-4 times and allow it to stand for 4-5 minutes so that the blood gets haemolysed.
- Centrifuge the haemolysed blood at 1500 rpm for forty minutes.
- Discard the supernatant carefully and transfer a drop of sediment onto a clean grease free slide and mix with an equal quantity of 0.1% Methylene blue or 0.2% Methyl green stain.

- Place a cover glass over it and examine under low and high magnifications.
- The whole sediment should be examined in the same manner before declaring the sample negative.
- In positive samples, blue or green stained elongated microfilariae can be observed. Note the length, width, presence or absence of sheath, cephalic space, caudal space, shape of the tail for identification. Haemolysed erythrocytes (ghosts) can be seen as dim outlined bodies.

Blood/Tissue fluid examination procedures:

Wet film method:

- It is used for the detection of live Trypanosomes.
- Place a small drop (~10µl) of fresh suspected blood over a clean glass slide.
- Cover with a cover slip and examine under low and high power of a microscope.

Staining methods:

- Used for detection of *Trypanosoma*, *Babesia*, *Theileria*, *Leishmania* and other parasites of animals.

Thin blood smear:

Giemsa's staining procedure

1. Clean 2 micro glass slides by rinsing them in 95% alcohol and wiping with a clean cloth.
2. Place a drop of fresh blood at the end of one slide, hold the other slide (spreader slide) having smooth edge at an angle of 40 45º in front of the drop over the first one.
3. Touch the drop of blood with slanted/spreader slide so that the blood is uniformly spread around the edge.

4. Draw the spreader/slanted slide quickly but very gently (without giving much pressure) over the length of the first one. The blood should be pulled behind the slide and not ahead of it. Allow to dry quickly.
5. The blood film is fixed in methanol for 2 minutes.
6. The slide is covered with sufficient dilute Giemsa stain (stock solution diluted 1:10 in distilled water, pH 7.2).
7. Allow it to stain for 30-40 minutes.
8. Wash quickly in distilled water thoroughly.
9. Air dry the slide and examine under low, high and oil immersion lens of the microscope.

Leishman/Wright staining procedure

- Steps 1 to 4, same as for Giemsa staining.

5. Place few (5-10) drops of stain to cover the unfixed fresh blood smear and allow it to stand for 2 minutes (not required to priorly fix in methanol since it will take place during staining process)
6. Add distilled water (pH 7-7.2) double the amount of the stain used and mix thoroughly by blowing with a pipette.
7. Allow it to stand for 10 minutes.
8. Wash thoroughly in running tap water and dry.
9. Examine under low, high and oil immersion lens of the microscope.

Lymph node fluid examination

Used for detection of lymphocytic stages of *Theileria* parasites present in lymph nodes and other protozoa like *Trypanosoma*

- Rinse a 2ml syringe with 20-22 gauze hypodermic needle in anticoagulant solution or physiological solution.
- Firmly hold the superficial lymph gland (prescapular, parotid, prefemoral) in between the index finger and middle finger of the left hand and puncture the gland with the needle on the syringe present in the right hand.
- Draw the lymphatic fluid in the syringe.
- Prepare a thick smear by placing a drop of fluid on a clean glass slide and air dry.
- Stain with 10% Giemsa solution as described for blood smear.
- Examine the lymphocytes for intracytoplsmic stage of *Theileria* under high power and oil immersion of a microscope.

Arthropod Diseases:

Diagnosis of MANGE INFESTATIONS by 10% KOH/ NaOH Method:

- ✓Take the scrapping as described above. Mix with 5-7ml of 10% KOH/NaOH in a centrifuge tube and boil the preparation.
- ✓ The material is then centrifuged at 1000 rpm for 3-5 minutes.
- ✓The supernatant is discarded and the whole sediment is examined microscopically.

Reagents used in parasitological examination

1. Potassium dichromate ($K_2Cr_2O_7$) solution (2.5%)	
Potassium dichromate	2.5g
Distilled water	100ml
2. Zinc sulphate solution	
$ZnSO_4\,7H_2O$	331g
Distilled water	1000ml
3. Lugol's Iodine (5%)	
Potassium iodide	10g
Iodine	5g
Distilled water	100ml
4. Sheather's sucrose solution	
Sucrose	500g
Distilled water	320ml
Phenol	6.5g
5. Carbol fuchsin	
Carbolic acid	2.5ml
Absolut alcohol	5ml
Basic fuchsin	0.5g
Distilled water	Upto 50ml

6. Acid alcohol	
Alcohol (70%)	95-99 parts
Conc. HCl	1-5 parts
7. Malachite green stain (5%)	
Malachite green	5g
Distilled water	100ml
8. Kinyoun's stain	
Basic fuchsin	4g
Phenol	8g
Alcohol (95%)	20ml
Distilled water	100ml
9. Sulphuric acid (10%)	
Sulphuric acid (conc.)	50ml
Distilled water	Upto500ml
10. Methyl blue (0.3%)	
Methyl blue	0.3g
Distilled water	100ml
11. Langeron's lactophenol	
Glycerine	2 parts
Distilled water	1 part
Phenol	1 part
Lactic acid	1 part

THANKS

भारत का राजपत्र
The Gazette of India

EXTRAORDINARY
No 29 NEW DELHI, FRIDAY, MARCH 20, 2009/29
Phalguna 1930
MINISTRY OF LAW AND JUSTICE
(Legislative Department)

The following Act of Parliament received the assent of President on the 20th March/2009 and is hereby published for general information

THE PREVENTION AND CONTROL OF INFECTIOUS AND CONTAGIOUS DISEASES IN ANIMAL ACT, 2009
NO 27 OF 2009

THE PREVENTION AND CONTROL OF INFECTIOUS AND CONTAGIOUS DISEASES IN ANIMAL ACT, 2009 NO 27 OF 2009

- It is an act to provide the prevention, control and eradication of infectious and contagious diseases affecting animals, for prevention of outbreak or spreading of such diseases from one state to another and to meet the international obligations of India for facilitating import and export of animals and animal products for matters connected therewith or incidental thereto

Chapter one (Sections 1 to 2)

PRELIMINARY

- **Section-1:** Short tilte and extent of commensement
- **Section 2:** Definitions:
- Animal:
  Cattle, buffalo, sheep, goat, yak, mithun, dog, cat, pig, horse, camel, ass, mule, poultry, bees and any other animal or bird as the Central Government may by notification specify
- Check post means a place established by the Directors to carry out checking of animals
- Competent officer means any person/ officer of the government notified by geoverment under section 17
- Compulsory vaccination means the vaccinations made mandatory under the provision of this act
- Scheduled diseases means the diseases named under provisions of this law
- Veterinarian means a person having recognized veterinary qualification who are allowed to treat animals under the existing laws

Chapter II (sections 3 to 19)
CONTRL OF SCHEDULED DISEASES

Section-3: Appointment of vety officers:

Section-4: Reporting of scheduled diseases is compulsory for

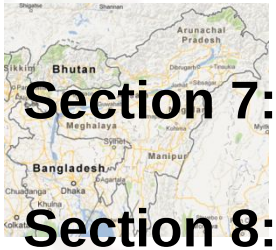
- Owner or any person, NGO, public bodies, village panchyayats, in charge of animals which he has a reason to believe to be infective in nature shall report to the nearest available veterinarian.
- The Veterinarian shall visit the area for reporting any outbreak to higher authority
- If the disease is a scheduled disease the Director will sent intimation to the Directors of the states which are in the immediate neighbourhood for taking preventive measures

Section-5: Segregation of animals:

- If the owner of person (animal in-charge) has a reason to believe to be infective in nature shall segregate the animal(s) away from other animals, prevent grazinfg at common place and prevent drinking from common sourc.
- All other animals shall be segregated by the Municipality, Panchyayat or local administration

Section 6: Notification of controlled and free areas:

- After notification in mass media all animals of the species in the controlled area must be compulsorily vaccinated against that disease along with other measures. No animals from the controlled area will be allowed to enter free areas unless it has been vaccinated.



Section 7: Prohibition of movement of animals from controlled area:

Section 8: Vaccination, marking and issue of vaccination certificate:

- Vaccine must be administered by a competent person as defined under the law
- Marking of the animal must be made by branding/ tattooing/ or ear tagging or in such other manner as the Director may deem fit (by special order)

Section 9: Contents of vaccination certificate:The vaccination certificate shall specify date of vaccination, dates of manufacture & expiry. The other content of the certificate is also notified under this law

Section 10: Entry and exit of animals from controlled and free area:

Section 11: Precautionary measures in controlled areas:

Section 12: Prohibition of markets, fairs exhibitions etc. in the controlled area

Section 13: Prohibition of bringing infected animals into markets and other places

No person will take out any animal (dead or alive), fodder, bedding or other in contact materials, carcass, skin or any other animal products, shall hold any animal market, animal fair, animal exhibition or carry out any activities which involves grouping of animals. No owner is allowed to bring animals to such events in case of declaration of an outbreak.

- **Section 14**: Check posts and quarantine posts:

for detention of animals believed to be or tested to be infected with scheduled diseases

- **Section 15:** Inspection and detention of animals at check posts and quarantine camps

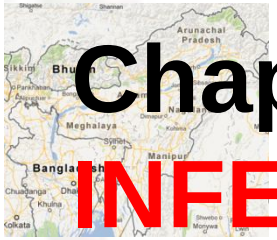
- **Section 16:** entry and exit of vaccinated animals into controlled and free area

- **Section 17:** Appointment of competent officers

- **Section 18:** Cleaning and disinfection of carriers

- **Section 19**: Power of entry and inspection:

Any Veterinary officer may enter and inspect any land, building or place, veswels or vehicles for the purpose of ensuring compliance of the provision of this acts or the rules by persons responsible for such compliance



Chapter-III (section 20 to 22)
INFECTED AREAS

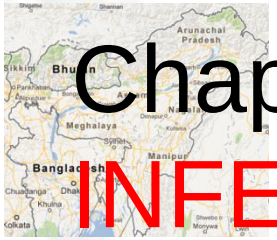
- **Section 20:** Declaration of infected areas:

The area where scheduled disease is occurring is to be declared as infected area

- **Section 21:** Effect of declaration of infected areas:
  - The owner should get their animals treated by a vet
  - The owner is to keep their animals in isolation

- **Section 22:** Denotification:

After resolving the area is to be denotified



Chapter-IV (sections 23 to 28)
INFECTED ANIMALS

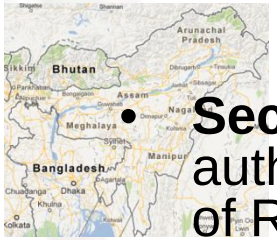
- **Section 23:** Segregation, examination and treatment of infected animals
- **Section 24:** Drawing of samples from animals: The vet have the authority of draw any sample from animals for detection of disease or vaccination status
- **Section 25:** Resort to Euthanasia for infected animals: If the Veterinary officer deems it necessary that an animal infected with scheduled disease can resort to euthanasia for preventing spread of the disease, to protect public health (in case of zoonotic disease) by an order in writing.
- **Section 26:** Disposal of carcass: Must be made as prescribed procedures
- **Section 27:** Power to perform Post mortem examination
- **Section 28:** Seizure and removal of certain animals: In cases of ownerless, owner not promptly complying to orders/ directives of Veterinarian in cases of scheduled diseases the Veterinarian has the power to seize and remove animals to a place of isolation.

Chapter- V (sections 29 to 34)

ENFORCEMENT OF PENALTIES

- **Section 29:** Enforcement of orders and recovery of expenses:
  - The owner or custodian of animal is responsible for carcass, diseased animal and he should promptly comply to orders at his own cost
  - Municipality or panchayat is responsible for compliance for ownerless animals at their own cost
  - The costs of any measures taken by the ordering authority for non compliance shall be recoverable from complying authority
  - The orders include: to give immediate information regarding animal disease, take necessary measures for prevention and spread to assist veterinary officers in discharge of their duties

- **Section 30:** Village officers etc are to assist**:** All municipal, panchayat or village offices and all officers of the rural and dairy development, revenue, agriculture, animal husbandry and veterinary departments of the state government are bound
  - To give immediate information to the veterinary officer and to the veterinarian having jurisdiction in the area regarding the prevalence or a scheduled disease amongst any animal or species of animals in the area
  - To take all necessary measures to prevent the outbreak or spread of any scheduled disease
  - To assist the veterinary officer and the veterinarian in the discharge of their duties or in the exercise of their posers under this act.



- **Section 31:** Penalty for issuing vaccination certificate without authority or administering defective vaccines: Punishable with a fine of Rs. 5000/- and in case of nonpayment imprisonment upto 1 month and in case of any subsequent offense fine is Rs. 10000/- of imprisonment upto 3 months

- **Section 32: Penalties**
- Obstructing the competent officers in performing duties is punishable with a fine upto Rs. 1000/- and or imprisonment upto 1 month and in case of subsequent offense penalty is Rs. 2000/- and or imprisonment upto 2 months

- **Section 33:** Penalty for placing infected animals/ carcass in rivers etc. : If a person places an infected carcass or parts thereof / diseased animals in rivers/ ponds/ lakes/ canal or any water bodies is punishable with a fine of Rs. 2000/- and or imprisonment upto 1 months and in subsequent offense penalty is Rs. 5000/- and or imprisonment upto 3 months

- **Section 34:** Offense by companies: The Director, Managing Director or the person responsible for running the company is responsible

Chapter VI (Sections 35)

PRECAUTIONARY MEASURES ON CAUSATIE ORGANISM ETC.

- Section 35: Prevention of escape of causative organism:

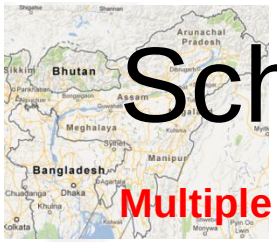
Every institution, laboratory, clinic engaged in manufacturing, testing, research with vaccines, sera, diagnostics, chemotherapeutics aimed at prevention and treatment of scheduled diseases should take adequate precautionary measures to ensure

  - Organism dies not escape or get released
  - To warn and to protect in case of any event of escape
  - The animals used in such experiments should be euthanized and disposed off

- Non compliance is punishable with fine of Rs. 20000/- and or imprisonment upto 6 months along with temporary suspension of manufacturing/ testing license for a period of 1 year

Chapter VII (Sections 36 to 45)
MISCELLANEOUS

- **Section 36**: Power to delegate: State Government have the power to delegate any person subordinate to it to have powers for enforcement of the section of the law
- **Section 37**: Officers to function under Govt control:
- **Section 38**: Power to amend the schedule
- **Section 39:** Power to issue directions: The central Govt has the power to issue directions for prevention, control an eradication of scheduled disease to state governments or other authorities.
- **Section 40**: Certain persons are to be public servants
- **Section 41**: Power to remove difficulties
- **Section 42**: Power of central Government to make rules
- **Section 43**: Power of state government to make rules: Form for Quarantine camp for releasing animals, manner of inspection and period of detention at check posts or in quarantine camp, vaccination and marking of animals and the forms including form for entry permit, any other matter in relation to existing rules
- **Section 44**: Laying of rules
- **Section 45**: Repeal and savings



Schedule diseases

Multiple Species Diseases

- Anthrax
- Aujeszky's disease
- Blue tongue
- Brucellosis
- Crimean Congo Haemorrhagic fever
- Echinococcosis hydatidosis
- Goot and mouth disease
- Heart water
- Japanese encephalitis
- Leptospirosis
- New world screw worm (Cochliomyia hominiorax)
- Ole world screw worm (Chrysomya bezziana)
- Paratuberculosis
- Q fever
- Rabies
- Rift valley fever
- Rinderpest
- Trichinellosis
- Tularemia
- Vesicular stomatitis
- West Nile fever

Schedule diseases

Cattle diseases

- Bovine anaplasmosis
- Bovine babesiosis
- Bovine genital campylobacteriosis
- Bovine spongiform encephalopathy
- Bovine tuberculosis
- Bovine viral diarrhoea
- Contagious bovine pleuropneumonia
- Enzootic bovine leucosis
- Haemorrhagic septicaemia
- Onfectious bovine rhinotracheitis/ infectious pustular vulvovaginitis
- Lunpy skin disease
- Malignant catarrhal fever
- Theileriosis
- Trichomonosis
- Trypanosomosis

Schedule diseases

- Sheep and goat disease
  - Caprine arthritis/ encephalitis
  - Contagious agalactia
  - Contagious caprine plueopneumnia
  - Enzootic abortion of ewes (Ovine chlamydiosis)
  - Maedi-Visna
  - Nairobi sheep disease
  - Ovine epididymitis (Brucella ovis)
  - Peste des petis ruminants
  - Salmonellosis (S. abortusovis)
  - Scrapie
  - Sheep pox and goat pox

Schedule diseases

- **Sheep and goat disease**
  - Caprine arthritis/ encephalitis
  - Contagious agalactia
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  - Scrapie
  - Sheep pox and goat pox

Schedule diseases

- **Swine diseases**
  - African swine fever
  - Classical swine fever
  - Nipah viral encephalitis
  - Porcine cysticercosis
  - Porcine reproductive and respirqtory symptoms
  - Swine vesicular disease
  - Transmissible gastroenteritis

Schedule diseases

- **Avian diseases**
  - Avain chlamydiosis
  - Avain infectious bronchitis
  - Avina infectious laryngotracheitis
  - Avain mycoplasmosis (m gallisepticum)
  - Avain mycoplasmosis (M synoviae)
  - Duck virus hepatitis
  - Fowl cholera
  - Fowl typhoid
  - Highly pathogenic avian influenza and low pathogenic avian influenza in poultry
  - Infectious bursal disease (Gumboro disease)
  - Marek's disease
  - Newcastle disease
  - Pullorum disease
  - Turkey rhinotracheitis

Schedule diseases

Lagomorph diseases

- Myxomatosis
- Rabbit haemorrhagic disease

Other diseases

- Camel pox
- Leishmaniosis

Thank You