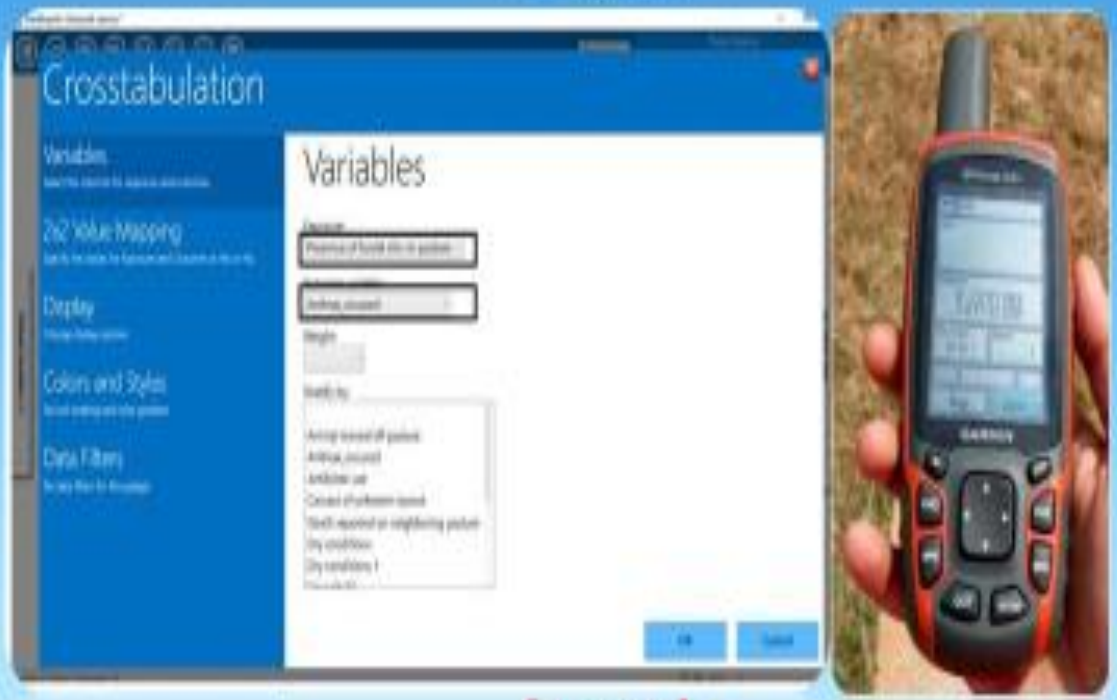
ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI)

Capacity Building Programme

On

Basic Veterinary Epidemiology and Economics of Animal Diseases



Sponsored

By

Indian Council of Agricultural Research, Krishi Bhawan, New Delhi,

under Network Project on

Outreach Programme on Zoonotic Diseases (OPZD)

Capacity Building Programme

On

Basic Veterinary Epidemiology and Economics of Animal diseases

31st January 2017 to 4th February 2017

Training Manual

Compiled and Edited

By

Dr. Md. Mudassar Chanda
Dr. V. Balamurugan
Dr. G.Govindaraj
Dr. Jagadish Hiremath
Mrs. Anusha Alamuri

Sponsored
By

Indian Council of Agricultural Research, Krishi Bhawan, New Delhi,
Under Network Project on
Outreach Programme on Zoonotic Diseases (OPZD)

Organized
By

ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), Post Box No. 6450,
Yelahanka, Bengaluru-560064

Acknowledgement

The constant support, encouragement and financial assistance from Indian Council of Agricultural Research, Krishi Bhawan, New Delhi, under Network Project on Outreach Programme on Zoonotic Diseases (OPZD) for sponsoring capacity building programme on "Basic Veterinary Epidemiology and Economics of Animal diseases" and providing opportunity to ICAR-NIVEDI collaborating centre for conducting such a workshop and training programme in the field of veterinary epidemiology and disease informatics are gratefully acknowledged.

भाकृ अनुप
ICAR

भारतीय कृषि अनुसंधान परिषद्
कृषि भवन, डा. राजेन्द्र प्रसाद मार्ग, नई दिल्ली-110001
Indian Council of Agricultural Research
Krishi Bhawan, Dr. Rajendra Prasad Road, New Delhi-110001

डा. हबिबर रहमान
उपमहानिदेशक (पशु विज्ञान)
Dr. H. Rahman
Deputy Director General
(Animal Science)

MESSAGE

I am glad to know that ICAR - National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), Bengaluru is organizing a Capacity Building Programme on "Basic Veterinary Epidemiology and Economics of Animal Diseases" during January 31 - 4 February 2017 under ICAR Network Project on "Outreach Programme on Zoonotic Diseases-(OPZD)".

Veterinary epidemiology and economics of animal diseases are important in carrying out disease surveillance, monitoring and impact analysis of livestock diseases and the workshop is designed to assess the economic loss and its projection.

I congratulate the organizers for taking the initiative for conducting training programme and will benefit the investigators from different OPZD collaborative centres in the field of epidemiology and to analyze the economic impact zoonotic diseases.

I wish the training programme a grand success.

(H. Rahman)

भारतीय कृषि अनुसंधान परिषद्
Indian Council of Agricultural Research
कृषि भवन : नई दिल्ली 110 001
Krishi Bhavan, New Delhi - 110 001
Telefax (O) : 011 23386668 Email: ashokkr.icar@gov.in, ashokakt@rediffmail.com

डा0 अशोक कुमार
Dr. Ashok Kumar
सहायक महानिदेशक (पशु स्वास्थ)
Assistant Director General (Animal Health)

MESSAGE

It is heartening to know that National Institute of Veterinary Epidemiology and Disease Informatics (ICAR-NIVEDI), Bengaluru is organizing a capacity building workshop on "Basic Veterinary Epidemiology and Economics of Animal diseases" during January 31 - 4 February, 2017 under ICAR Network Project "Outreach Programme on Zoonotic Diseases".

A major role of veterinary epidemiology and economics of animal diseases is involved in investigation, survey and descriptive studies. I am sure that the training Programme pertaining specific to various epidemiological studies helps the principal investigators and co-principal investigators from different OPZD collaborative centres to analyze the zoonotic diseases and also will provide a right platform for the economic impact analysis.

I wish the workshop all the success and compliment the organizers for selecting the appropppriate theme for deliberations.

(Ashok Kumar)

Dr. B. P. Mishra,
Joint Director (Research) and Project Coordinator (OPZD)
ICAR-IVRI, Izatnagar, Bareilly, U.P.

MESSAGE

It gives me great pleasure to know that National Institute of Veterinary Epidemiology and Disease Informatics (ICAR-NIVEDI), Bengaluru is organizing a Capacity building workshop on "Basic Veterinary Epidemiology and Economics of Animal diseases" during January 31st - 4th February, 2017 under ICAR Network Project on "Outreach Programme on Zoonotic Diseases"

Epidemiology is to know the distribution and determinants of health related states or events in specified populations and the application of this study to control health problems. Typically one tries to find associations between risk factors (exposures) and diseases by studying the occurrence of diseases in populations and to enlighten the scientists to strengthen in the area of epidemiology and disease informatics.

Best wishes for the workshop.

(B. P. Mishra)

भाकृअनुप–राष्ट्रीयपशुरोगजानपदिकएवंसूचनाविज्ञानसंस्थान

ICAR–National Institute of Veterinary Epidemiology and Disease Informatics, रामगोंडनहल्ली, येलहंका, बेंगलुरू – 560064

Ramagondanahalli, Post Box No: 6450, Yelahanka, Bengaluru – 560064

(ISO 9001-2008 certified)

Dr. Parimal Roy,
Director

From the Director Desk…

I am pleased that ICAR- National Institute of Veterinary Epidemiology and Disease Informatics (ICAR-NIVEDI), Bengaluru is organizing a capacity building workshop on "Basic Veterinary Epidemiology and Economics of Animal diseases" during January 31st-4th February, 2017 sponsored by ICAR under Network Project "Outreach Programme on Zoonotic Diseases".

Epidemiology is the study of diseases in populations and useful for disease management. It is based on observing differences and similarities between diseased and non-diseased animals in order to understand what factors are playing role in increasing or reducing the risk of diseases. NIVEDI, under ICAR has a mandate to conduct R & D in the field of veterinary epidemiology and animal disease surveillance for the entire country. Its role is extremely pivotal in developing models for risk analysis, animal disease forecasting, forewarning, need based diagnostics and economic impact analysis of the diseases. As technology dissemination and HRD process, the Institute has been conducting plethora of training programmes related to epidemiology, economic impact, development of economic analysis modules, research methodologies, sampling frame and disease diagnosis, application of ELISA, offline and online software for various stakeholders associated with animal health especially scientists and veterinary professionals drawn from various parts of the country as part of capacity building in the area. I hope this workshop will also help all the participants from different centres and strengthen the capabilities in areas of epidemiology.

I wish the participants a pleasant stay in green city and I wish the workshop a great success.

(Parimal Roy)

भाकृअनुप–राष्ट्रीयपशुरोगजानपदिकएवंसूचनाविज्ञानसंस्थान
ICAR–National Institute of Veterinary Epidemiology and Disease Informatics
रामगोंडनहल्ली, येलहंका, बेंगलुरू – 560064
Ramagondanahalli, Post Box No: 6450, Yelahanka, Bengaluru – 560064
(ISO 9001-2008 certified)

Dr. V.Balamurugan,
Senior Scientist,
Course Director

Understanding the basic epidemiological concepts helps the researchers to understand accurate status of the disease and risk factors associated with disease. Epidemiological data collection and analysis is the basis for formulating prevention and control policy as well as detecting the new emerging or re-emerging disease/pathogens. In case of zoonotic diseases the accurate burden of the disease in animals and risk of humans getting infection needs to be ascertained. Outbreaks investigations in the field starts with careful observation of clinical signs and confirmation is done by laboratory testing of clinical samples and disease diagnosis. GPS and GIS are employed to better understanding of epidemiology of livestock disease at macro level. These tools are very important for developing risk maps and forecasting models of livestock disease so as to take timely preventive measures in order to minimize the morbidity and mortality. Further, the livestock diseases have numerous impacts like productivity loss, loss of income, prevention and control costs etc., In animals, more specifically, milk loss, loss of drought power, opportunity cost of labor, and treatment cost are important, where as in human, the house hold productivity loss and treatment cost is highly important. In overall, to assess the real cost of the disease, economic analysis of the disease is important, more so in zoonotic diseases like brucellosis, leptospirosis, parasitic zoonosis etc., Therefore, it is important to make the socio-economic impact assessment of the disease to better devise the targeted strategies for effective prevention and control measures of the disease in the long run.

This capacity building training programme is aimed at refreshing and strengthening skills of researchers in the fields of epidemiological data collection & analysis and to assess the economic impact associated with livestock diseases. I hope that, this training course, will strengthen the participant's skills in collection and analysis of epidemiological data. The training will also refresh participants skills in sampling techniques and sample size estimation/requirement for epidemiological studies, epidemiological data collection (host, agent and environment) and tools required for data collection (using GPS), risk factors identifications (using questionnaire) for livestock diseases, generation of various epidemiological Maps (using GIS), etc., including impact methodologies for the zoonotic diseases and participants will be able to assess the economic loss and its projection on their respective working diseases.

I extend warm welcome to our ICAR network project on Outreach Programme on Zoonotic Diseases (OPZD) coordinating and collaborating centres' research participants and wish them a memorable stay in this garden city and enriching their knowledge along with their learning experiences at ICAR-NIVEDI and also to leave beside difficulties experienced if any.

With warm regards,

(V.Balamurugan)

Historical Background

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

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

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

On 9thJanuary'2015, NIVEDI's newly constructed administrative building and Biosafety Laboratory (BSL-2) was dedicated to the nation by Shri Radha Mohan Singh, Hon'ble Union Minister for Agriculture, New Delhi in the presence of Shri D.V. Sadananda Gowda, Hon'ble Minister of Law and Justice, GOI and Shri T. B. Jayachandra**,** Hon'ble Minister for Law, Justice & Human Rights, Parliamentary Affairs and Legislation and Animal Husbandry, Govt. of Karnataka and Dr. S. Ayyappan, Secretary DARE and Director General, ICAR. The centralised administrative and laboratory complex of the institute is located in a sprawling campus at Yelahanka, Bengaluru.

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

(i) Vision

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

(ii) Mission

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

(iii) Focus

- Improving disease monitoring and surveillance through development of penside diagnostics
- Risk assessment for occurrence of economically important animal diseases
- Adapting strategies to improve animal disease data quality
- Understanding the threat from animal diseases in the background of climate change and globalization
- Developing early warning system and disease modeling/ forecasting
- Understanding economic impacts of animal diseases and the management strategies
- Promoting innovations and improving human resource capacity
- Fostering linkages and collaborations with public and private, national and international organizations
- Improving knowledge management system

(iv) Mandate of Institute

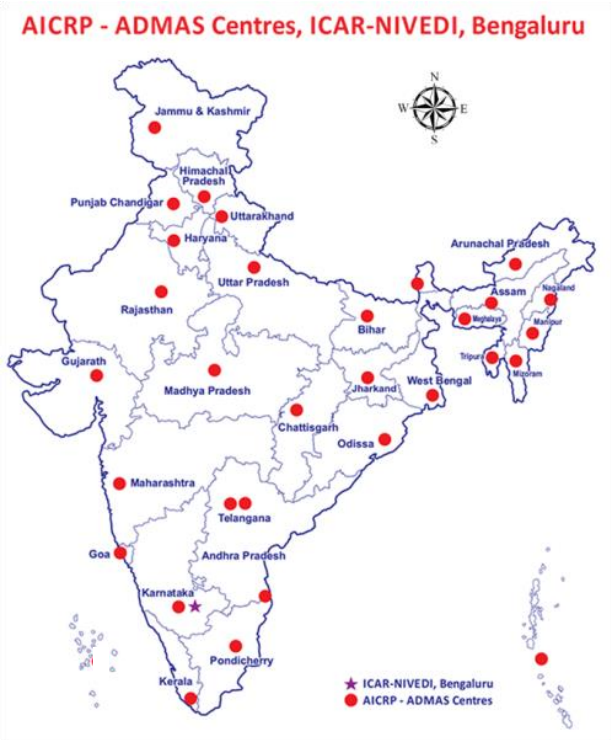
- Epidemiology, informatics and economics of animal diseases including zoonosis
- Surveillance, forecasting and forewarning for management of animal diseases including Zoonosis
- Repository and Capacity Development

(v) AICRP on ADMAS

All AICRP collaborating units are extensively working on animal disease diagnosis, outbreak investigation, disease reporting, pathogen characterization and mapping etc., with major focus on bacterial (Brucellosis, Leptospirosis, Mastitis, Haemorragic Septicaemia, Anthrax, Black Quarter, Enterotoxaemia,), viral (Infectious Bovine Rhinotracheitis, Bluetongue, Classical Swine Fever, *Peste des Petits Ruminants* and Sheep and Goat Pox) and parasitic (Trypanosomosis, Theileriosis, Babesiosis, Fascioliosis and Amphisomiosis) diseases of economic importancewith the following mandates.

Mandates of AICRP on ADMAS

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



ICAR Network project on Outreach Programme on Zoonotic Diseases

ICAR- NIVEDI Centre, Bangalore

<u>Present Components for ICAR- NIVEDI</u>: Leptospirosis, Anthrax and Toxoplasmosis

1. Principal Investigator: **Dr. V.Balamurugan**

Senior scientist & Principal Investigator (w.e.f. 6.9.2014)

Co-PI (up to 5.9.2014)

Dr. (Mrs.) RAJESWARI SHOME

Principal Scientist, (2009 to 5.9.2014)

2. Co- Principal Investigators : (i) **Dr.P.P.Sengupta**

Principal scientist (w.e.f. 6.9.2014)

(ii) **Dr.R.Sridevi**

Scientist (w.e.f. 6.9.2014)

(iii) **Dr. M.Nagalingam**

Scientist (up to 27.7.2014)

(iv) **Dr. Satish .B. Shivachandra**

Scientist (w.e.f. 28. 3. 2016)

(v) **Dr. Md. Mudassar Chanda**

Scientist (w.e.f. 28. 3. 2016)

Objectives:

1. To monitor/determine the prevalence of Leptospirosis, Toxoplasmosis and Anthrax among humans, livestock and pets.
2. To determine the incidence of active infection among the risk groups.
3. Epidemiological analysis of disease in livestock and humans and anthrax outbreaks in domestic and wildlife in India and Molecular characterization of circulating *Bacillus anthracis* strains in India.

Technical details

- ✓ Collection of samples from livestock /pet and human closely associated with animals including veterinarians, Para-veterinarians etc.
- ✓ Screening of samples for above mentioning diseases by using commercially available kit/gold standard tests.
- ✓ Attending the field cases associated with abortion/reproduction disorder for collection of samples and subsequent screening for disease diagnosis.
- ✓ Subsequent monitoring of the zoonotic potentiality of the diseases.

Highlights of the project work

a. Diagnostic services provided to farmers and risk group personnel.
b. Generated baseline epidemiological data, which will help to know the prevalence and distribution of zoonotic diseases associated with human and livestock.
c. Conducted trainings to research and veterinary officers on various diagnostic tests.
d. Multiplex PCR developed helped in identification of the active infection in the animals and humans by detection of leptospira genomic DNA as well as detection of antibodies by MAT in the serum samples.

Significant Achievements of OPZD project so far

Brucellosis	• Burden in livestock and humans including risk group documented. • Epidemiology and risk analysis of human brucellosis in 1175 human population. • Concurrent occurrence of Brucellosis and national survey on zoonotic diseases conducted • Characterization of the Brucella isolates
Listeriosis	• Standardization of LLO based I-ELISA for Listeriosis • Development of Listeriolysin-O based lateral flow assay (LFA) and evaluation.
Leptospirosis	• Documentation of disease burden in livestock and humans including risk group. • Development of Multiple PCR for detection and differentiation of pathogenic leptospira. • 79.10% - Seropositivity in case of bovine abortion and reproductive disorders. • 38% - Seropositivity was observed in human PUO cases.
Toxoplasmosis	• 42.8% - Seropositivity in human toxoplasmosis in PUO cases and Neurological disorder cases..
Anthrax	• Facilities were developed for isolation of *Bacillus anthracis* from clinical specimens. Samples from Odisha outbreaks were screened by staining, biochemical and cultural techniques and PCR and provided confirmatory diagnosis as and when for taking appropriate control measures.
Concurrent infections	• Concurrent occurrence of Brucella, Leptospira and Listeria were documented. • Seropositivity for Leptospirosis and Toxoplasmosis were observed.

Organizing Committee for capacity Building Programme

Chief Patron: Dr. Parimal Roy, Director, ICAR-NIVEDI, Post Box No. 6450, Yelahanka, Bengaluru-560064, Karnataka, India. Phone: +91-80-23093111(O); Fax: +91-80-23093222; Mobile: 9884472767; E-Mail:parimalroy580@gmail, com; director.nivedi@icar.gov.in

Course Advisor: Dr. P.P.Sengupta, Principal Scientist, ICAR-NIVEDI, Bengaluru

Course Director: Dr.V.Balamurugan, Senior Scientist, ICAR-NIVEDI, Post Box No. 6450, Yelahanka, Bengaluru-560064, Karnataka, India. Phone: +91-80-23093100 / 23093111(O) Mobile: 9481807438; E-Mail: 1; b.vinayagamurthy@icar.gov.in

Course Coordinator: Dr. Md. Mudassar Chanda, Scientist, ICAR-NIVEDI, Bengaluru

Course Co-coordinators

Dr. Jagadish Hiremath, Scientist, ICAR-NIVEDI, Bengaluru

Dr .G.Govindaraj, Senior Scientist, ICAR-NIVEDI, Bengaluru

Dr. K.P.Suresh, Senior Scientist, ICAR-NIVEDI, Bengaluru

Dr. Sathish B. Shivachandra, Senior Scientist, ICAR-NIVEDI, Bengaluru

Technical Assistants

Mrs. Anusha Alamuri, SRF, OPZD , Project

Dr. Nayana Kumara
Ms. Bhavana G. B.

Committee for Training Programme

Committee	Members
Registration, Inauguration and valedictory committee	Dr. GB Manjunatha Reddy Dr. P.Krishnamoorthy Dr. M.Nagalingam Dr. Siju Susan Jacob
Technical Course Manual Publication committee	Dr. V.Balamurugan Dr. Md. Mudassar Chanda Dr. Jagadish Hiremath, Dr .G.Govindaraj
Transportation committee	Dr. A. Prajapathi Dr. Manjunatha Reddy Dr. Md.Mudassar Chanda Dr. Govindaraj Mr. Rajeeva lochana
Accommodation committee	Dr.V.Balamurugan Dr. Md.Mudassar Chanda Mr. Rajeeva lochana Mr. Ramu
Food and tea committee	Mr. R.K.Babu Dr. Jagadish Hiremath Dr. P.Krishnamoorthy Mr. Rajeeva lochana
Financial Committee	Dr. V.Balamurugan Mr. Rajeeva lochana Mr. Babu R.K
Associated Staffs	Mrs. Anusha Alamuri Ms.Taniya Goshal Mr. Bharat kumar Dr. Nayana kumara Ms. Bhavana

Faculties Involved

Course Patron	**Dr. Parimal Roy**	**Director, ICAR-NIVEDI**
Course Director	**Dr. V.Balamurugan**	**Senior Scientist , ICAR-NIVEDI**
Course Advisor	**Dr. P.P.Sengupta**	**Principal Scientist , ICAR-NIVEDI**
Course Coordinator	**Dr.Md. Mudassar Chanda**	**Scientist, ICAR-NIVEDI**
Course co-Coordinator	**Dr. Jagadish Hiremath**	**Scientist, ICAR-NIVEDI**
	Dr. G. Govindaraj	**Senior Scientist, ICAR-NIVEDI**
	Dr. K.P. Suresh	**Senior Scientist, ICAR-NIVEDI**
Faculties	**Dr. D. Hemadri**	**Principal Scientist , ICAR-NIVEDI**
	Dr. S.B. Shivchandra	**Senior Scientist, ICAR-NIVEDI**
	Dr. P. Krishnamoorthy	**Scientist, ICAR-NIVEDI**
	Dr. M. Nagalingam	**Scientist, ICAR-NIVEDI**
	Dr. G.B. Manjunatha Reddy	**Scientist, ICAR-NIVEDI**
	Dr. Siju Susan Jacob	**Scientist, ICAR-NIVEDI**
Field and Laboratory Support	**Mrs. Anusha Alamuri**	**SRF, OPZD project**
	Mr. K. Bharat Kumar	**Young Professional I**
	Ms.Taniya Goshal	**Young Professional I**
	Dr. Nayana kumara	**Young Professional II**
	Ms. Bhavana	**Young Professional I**

Training schedule (31st January to 4th February 2017)

Basic Veterinary Epidemiology and Economics of Animal diseases

Day	Time	Topic	SPEAKER
31.1.2017	8.30-10 AM	Registration	
	10-11 AM	Inauguration	
	11-11.30 AM	Overview of the course and Pre-training evaluation	Dr. V.Balamurugan and Dr. Md. Mudassar Chanda
	11.30-12.15 PM	Basic epidemiological concepts	Dr. Siju Susan Jacob
	12.15-1 PM	Epidemiological surveys, monitoring and surveillance	Dr. G.B. Manjunathareddy
	1-2 PM	**Lunch**	
	2-2.45 PM	Types of Research: Experimental Vs Observational (Theory)	Dr. V.Balamurugan and Dr. M.Nagalingam
	2.45-3.30 PM	Measures of disease (Theory)	Dr. Sathish B. Shivchandra Dr. Jagadish Hiremath
	3.30-5 PM	Sampling technique and sample size estimation (Theory & Practical)	Dr. K.P.Suresh
1.2.2017	10-10.45 AM	Sample collection, packaging & transportation	Dr. P.Krishnamoorthy
	10.45-11.30 AM	Diagnostic tests –Epidemiological View	Dr. M.Nagalingam
	11.30-12 noon	Outbreak Investigation (Theory)	Dr. D.Hemadri
	12-1 PM	Development of questionnaire (Theory)	Dr.J.Hiremath
	1-2 PM	**Lunch**	
	2-3 PM	Creating questionnaire database in Epi-Info (Practical)	Dr. Md.Mudassar Chanda
	3-4 PM	Entry of questionnaire data in to Epi-Info (Practical)	Dr. Md.Mudassar Chanda
	4-5 PM	Analysis of questionnaire data using Epi-Info (Practical)	Dr. Md.Mudassar Chanda, Dr.J.Hiremath
2.2.2017	10-11 AM	Outbreak Investigation: Use of GPS (Practical)	Dr. Md.Mudassar Chanda, Dr.J.Hiremath
	11-12 PM	Introduction to GIS (Theory)	Dr. Md.Mudassar Chanda

	12-1 PM	Use of GIS in spatial epidemiology (Theory)	Dr. Md.Mudassar Chanda
	1-2 PM	Lunch	
	2-3PM	Display of spatial data: Chloropeth maps (Demonstration) Display of spatial data: Point maps (Demonstration)	Dr. Md.Mudassar Chanda
	3-4.30 PM	Display of spatial data: Chloropeth and Point maps (Practical)	Dr. Md.Mudassar Chanda
	4.30-5 PM	Preparation for field visit on next day	Dr. Md.Mudassar Chanda
3.2.2017	8.30 to 1 PM	**Field Visit for epidemiological collections**	Dr. V.Balamurugan, Dr. Dr. Md.Mudassar Chanda,Dr. Manjunatha reddy GB
	1-2 PM	**Lunch**	
	2-3 PM	Impact on livestock diseases- Introduction	Dr. G.Govindaraj
	3-4 PM	Identification of various economic impact parameters for livestock diseases-a discussion on participatory mode	Dr. G.Govindaraj
	4-5 PM	Methodologies for impact assessment of Livestock diseases and loss projection methods	Dr. G.Govindaraj
4.2.2017	10-11 PM	Economic loss assessment due to Livestock diseases and loss projection methods using data collected by the participants	Dr. G.Govindaraj
	11-1 PM	Entry of field questionnaire data in Epi-Info software and analysis. Analysis of data of participants and any issues related to the training.	Dr. Md.Mudassar Chanda, Dr. G.Govindaraj, Dr. V.Balamurugan
	1-2 PM	**Lunch**	
	2-3 PM	Post-training evaluation and Feedback	
	3-5 PM	Valedictory	

Contents

Basic Epidemiological Concepts

Siju Susan Jacob, Scientist,
ICAR – National Institute of Veterinary Epidemiology and Disease Inforamtics
Yelahanka, Bengaluru, Karnataka 560064

Epidemiology: The word epidemiology comes from Greek words '**epi**', meaning on or upon, '**demos**', meaning people and '**logos**', meaning the study of. The basis for any epidemiological investigations is the assumption that disease does not occur in a random fashion because one of the main objectives of epidemiologist is to identify causal relationships between potential risk factors and outcomes such as disease or productivity losses. Both types of losses are assumed to be influenced by multiple, potentially interacting factors. Epidemiological investigations focus on general population and disease aspects as well as on causation of the disease.

Definition of Epidemiology:

'Epidemiology is the study of distribution and determinants of health-related states or events in specified populations and the application of this study to the control of health problems.'

Key terms in this definition reflect some of the important principles of epidemiology.

Study: Epidemiology is the basic science of public health. It is a highly quantitative discipline based on principles of statistics and research methodologies. Epidemiology is data-driven and relies on a systematic and unbiased approach to the collection, analysis and interpretation of data. Basic epidemiologic methods tend to rely on careful observation and use of valid comparison groups to assess whether what was observed, such as the number of cases of disease in a particular area during a particular time period or the frequency of an exposure among persons with disease, differs from what might be expected.

Distribution: Epidemiology is concerned with the frequency and pattern of health events in a population. Frequency refers not only to the number of health events, but also to the relationship of that number to the size of the population. The resulting rate allows epidemiologists to compare disease occurrence across different populations.

Pattern refers to the occurrence of health-related events by time, place and person. Time patterns may be annual, seasonal, weekly, daily, hourly or any other breakdown of time that may influence disease occurrence. Place patterns include geographic variation, urban/rural differences, and location. Personal characteristics include demographic factors which may be related to risk of illness, injury, or disability such as age, sex and socio-economic status, as well as behaviors and environmental exposures.

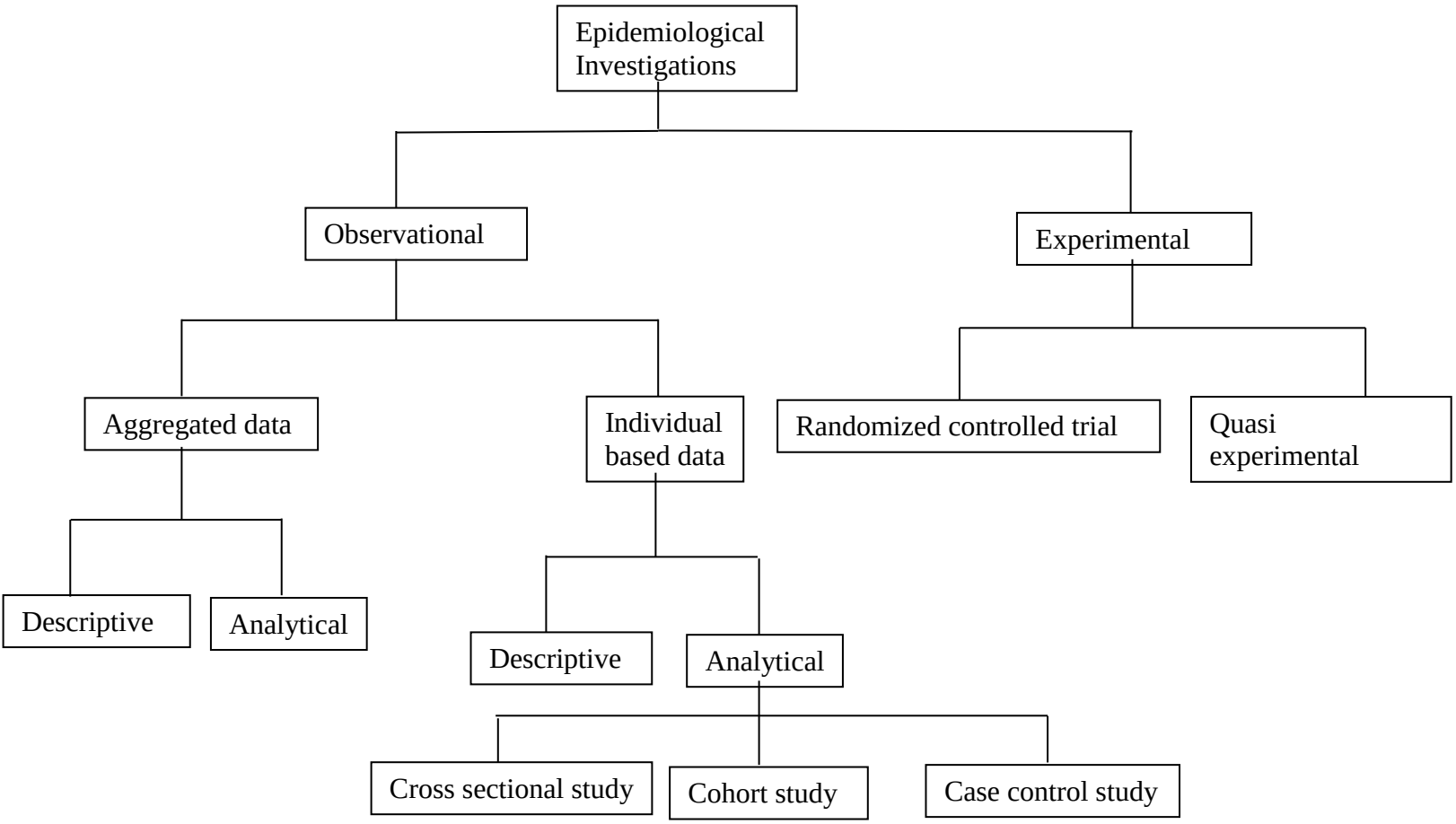
Determinants: Epidemiologists search for causes or factors that are associated with increased risk or probability of disease. A *determinant* is any factor or variable that can affect the frequency with which a disease occurs in a population. Determinants can be broadly classified as being either intrinsic or extrinsic in nature. Intrinsic determinants are physical or physiological characteristics of the host or disease agent which are generally determined genetically. Extrinsic determinants are normally associated with some form of environmental

influence on the host or disease agent. They may also include interventions made by man into the disease process by the use of drugs, vaccines, dips, movement controls and quarantines.

Health-Related States: Early epidemiological study focused solely on infectious diseases. Today epidemiology studies a variety of health-related events, which includes chronic disease, environmental problems, behavioral problems, and injuries, in addition to infectious diseases.

Populations: A population can be defined as the complete collection of individuals that have some particular characteristic(s) in common. Depending on the characteristic(s) being considered, a population can be very large or very small. For example, one may wish to study a particular disease in a particular cattle population in a particular country. That cattle population could consist of: All the cattle in the country or all the dairy cattle in the country or all the dairy cattle of a certain breed in the country etc.

Types of Epidemiological Investigations:



Observational study

In observational study, the distribution of an exposure and/or an outcome is examined without any attempt by the investigator to influence them.

Experimental study

A study designed to test a hypothesis by modifying an exposure within the study population.

Aggregated data

Data concerning exposures and outcomes in which the unit of analysis is the population rather than individual animals.

Example for aggregated data is examining the data on the overall incidence of bovine spongiform encephalopathy cases in different countries rather than in individual animals.

Individual-based data

Data concerning exposures and outcomes derived from individual animals within the population.

Randomised controlled trial

It is the study in which individual animals are randomly assigned to receive the exposure under investigation or to be a control who does not receive the exposure. For example, in a randomised controlled trial to investigate the effect of kennel cough vaccine in dogs coming to a boarding kennel, dogs were randomly assigned to receive the vaccine or to be a control (no vaccine).

Quasi experimental study

In this study the investigator does not have full control over the allocation of the exposure. For example, a comparison of the incidence of bovine tuberculosis before and after the introduction of a control programme would be a quasi-experimental study.

Cross-sectional study

In cross sectional study, the prevalence of an exposure and/or an outcome is measured in a given population at a specified point in time. The data may be analysed to find an association between the exposure and the outcome. For example, prevalence of *Mycobacterium paratuberculosis* infection in a particular herd could be determined by a descriptive cross-sectional survey. If data on possible risk factors for paratuberculosis infection are collected from the same animals at the same time then they could be used in an analytic study to look for associations between the risk factors and the disease.

Descriptive epidemiology: It can be used to measure the burden of disease within a population. The 5W's of descriptive epidemiology include, **W**hat = health issue of concern, **W**ho = animals, **W**here = place, **W**hen = time and **W**hy/how = causes, risk factors, modes of transmission.

For example, descriptive epidemiology can be used to examine changing pattern of pre-weaning mortality in a particular country over the last 10 years, or to describe differences in the prevalence of hip dysplasia between various dog breeds, or to compare the incidence of bovine tuberculosis in different countries.

Analytical epidemiology:

With analytical epidemiology, risk factors for a disease or an outcome of a disease can be investigated. In analytical epidemiology, statistical analysis of epidemiological data will be attempted to establish relationships between causative factors and incidence of disease.

Cohort study

In cohort study one or more groups of individual animals are followed up over a period of time to determine the frequency of a particular outcome.

For example, in a cohort study of the effect of the bovine spongiform encephalopathy (BSE) status of the mother cow on the risk of BSE in her offspring, calves from diseased and non-diseased cows were followed-up until disease occurrence. The frequency of BSE could then be compared between calves having a dam with or without BSE.

Case-control study

In case control study individuals with and without the outcome of interest are identified. Their status with respect to exposures of interest is then determined in order to look for associations between these exposures and the outcome of interest.

For example, a case-control study can be used to determine risk factors for bovine tuberculosis in cattle at the herd level. For this identifying the herds with and without bovine tuberculosis and obtain information concerning exposures of interest (purchase, neighbours, wildlife) is required. After that analysis of the data to find out whether the presence of TB in neighbouring herds or frequent purchase of cattle was associated with bovine tuberculosis presence in the herd.

Measures of health

A fundamental task in epidemiology is to quantify the occurrence of disease. This can be done by counting the number of affected animals. Quantifying the levels of disease in a population is important since it allow to determine the economic importance of diseases and to prioritize the resources for disease control activities. The proportion and ratio are the key terms required for the quantification of diseases in a population:

A **proportion** is a fraction in which the numerator is included in the denominator. In a herd of 100 cattle over a 12-month period if 58 animals are identified as diseased, then the proportion diseased animals is $58 \div 100 = 0.58 = 58\%$.

A **ratio** defines the relative size of two quantities expressed by dividing one (numerator) by the other (denominator). The odds of disease (a ratio) in the above herd of 100 cattle can be calculated as 58:42 or 1.4 to 1.

Measures of health include the following terms:

The term **morbidity** is used to refer to the extent of disease or disease frequency within a defined population. Morbidity can be expressed as either **prevalence** or **incidence**.
Prevalence refers to the number of cases of a given disease or attribute that exists in a population at a specified point in time. Two types of prevalence are (1) **point prevalence**: equals the number of disease cases in a population at a single point in time (2) **period prevalence**: equals the proportion of the population with a given disease or condition over a specific period of time.

Incidence measures how frequently initially susceptible individuals become disease cases as they are observed over time. An incident case occurs when an individual changes from being susceptible to being diseased.

Attack rates are usually used in outbreak situations where the period of risk is limited and all cases arising from exposure are likely to occur within the risk period. Attack rate is defined as the number of cases divided by the number of animals exposed.

Secondary attack rates are used to describe infectiousness. The assumption is that there is spread of an agent within an aggregation of individuals and that not all cases are a result of a common-source exposure. Secondary attack rates are the number of cases at the end of the study period less the number of initial (primary) cases divided by the size of the population that were initially at risk.

Mortality is an example of incidence where death is the outcome of interest. Cause specific mortality risk is the incidence risk of fatal cases of a particular disease in a population at risk of death from that disease.

Case fatality rate refers to the incidence of death among animals which develop the disease.

Case fatality risk reflects the prognosis of disease among cases, while mortality reflects the burden of deaths from the disease in the population.

Epidemiological Surveys, Monitoring and Surveillance

Dr. G.B. Manjunatha Reddy
ICAR – National Institute of Veterinary Epidemiology and Disease Inforamtics
Yelahanka, Bengaluru, Karnataka 560064

The disease investigation starts with relevant data collection. The investigations either qualitative or quantitative in nature which are main components of epidemiology.

1. *Qualitative investigations*
    - The natural history of disease: Ecology and causal factors
    - Causal hypothesis testing
2. *Quantitative investigations*
    - Surveys
    - Monitoring and surveillance
    - Studies
    - Modelling
    - Evaluation (Biological & Economic)

Surveys:

A survey is an examination of an aggregate o units or group of animals (Kendall and Bucklan, 1982). An epidemiological survey is conducted in an epidemic or disease outbreak to discover the source of an infection, the means transmission, and the circumstances that gave rise to the disease. The findings are used to devise ways of preventing the disease from spreading. The surveys are undertaken on a sample of the population, whereas the census examines the total animal population.

There are two types of surveys

1. *Cross sectional survey:* Recording the events occurring at a particular point in time.
2. *Longitudinal survey:* Recording of the events over a period of time

There are other surveys like if the survey is conducted with the purpose of diagnosis of disease then it is called screening. Again which may be of mass screening (investigation of whole population) or strategic screening (targeted animals in certain areas where the disease have been reported or recorded).

The data are collected in different ways, among them the three major methods/ways are:

1. *Observation:* Clinical examination, diagnostic imaging, post-mortem examination etc.,
2. *Questionnaires:* Directly or by interviews
3. *Documentary sources:* Clinical records, laboratory records, production records etc.,

The data derived from the first two methods is called as primary data and the data derived from the third method is called as secondary data.

Steps involved in planning and conducting a survey:

- Early planning
- Choice of examination methods

- Staff and training: In a small study the investigator/s himself may do all the work, but in large surveys they need supporting staff.
- Sample size.
- Sampling methods
- Recruiting subjects
- Response rates
- Analysis

Monitoring:

Monitoring is the making of routine observations on health, productivity and environmental factors and the recording and transmission of these observations.

Eg: Milk yield recording, Meat inspection at abattoirs etc.,

Surveillance:

Surveillance is more intensive form of data recording than monitoring having three distinct elements.

1. Gathering, recording and analysis of data
2. Dissemination of information to interested parties
3. Action for control of disease.

The types of surveillance are as follows:

1. Disease surveillance: Occurrence and spread of disease
2. Epidemiological surveillance: General description of surveillance
3. Sentinel surveillance: Keep watch on a disease Eg: Stray dogs for canine parvovirus infection.
4. Sero-surveillance: Identification of patterns of current and past infection using serological tests.
5. Passive surveillance: Examination of only clinically affected animals, continue monitoring of existing diseases. Routinely collected data laboratory diagnoses, routine meat inspection and statutory notification of disease so it is taken with an intension of acting on its findings.
6. Active surveillance: Examination of both clinical and apparently healthy animals, monitoring of specific disease.
7. Targeted surveillance: Collecting specific information (similar to active surveillance) Eg: BSE of fallen stock
8. Scanning surveillance: Continuous watch over endemic disease (similar to passive surveillance)
9. Syndromic surveillance: Based on set of clinical sings.

Goals of surveillance:

- Rapid detection of disease outbreaks.
- Early identification of disease problems.
- Monitoring of spread of the disease.
- Assessment of health status of a defined population.

- Definition of priorities for disease control and prevention.
- Identification of new and emerging diseases.
- Evaluation of disease control programmes.
- Provision of information to plan and conduct research.
- Confirmation of absence of a specific disease.

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Study Designs In Epidemiology

Dr. V.Balamurugan and Dr. M. Nagalingam

ICAR – National Institute of Veterinary Epidemiology and Disease Inforamtics
Yelahanka, Bengaluru, Karnataka 560064

Understanding disease causation, estimating the quantitative effects of the various component causes that contribute to the occurrence of disease and measuring the burden of disease including identification of the risk factors are the main components underlying epidemiological studies. A study design is a plan for selecting study subjects and for obtaining data about them. It allows the investigator to translate the conceptual hypothesis into an operational one. There are three main epidemiological study designs namely descriptive, analytical and experimental.

I. Descriptive study design

This study design includes case report, case series and descriptive studies based on rates. Descriptive study designs generally create new hypotheses which can further lead to analytical studies.

i. Case report

Case report describes and interprets an individual case, often written in the form of a detailed story. Usually report may reflect an important variation of the disease or condition. Generally case reports are the first line of evidence. It can help in bringing in new issues and ideas.

ii. Case series

Case series is coherent set of cases which identify common features among multiple cases and describes patterns of variability among them.

iii. Descriptive studies based on rates

Measures of disease frequency such as prevalence, incidence, morbidity, mortality etc., are used to quantify the burden of the disease in a population.

II. Analytical study design

It includes Cross sectional, case-control, cohort and ecological studies

i. Cross sectional

It is a simplest form of an observational study. It is based on single examination or a cross section of population at one point in time and these things can be applied to the entire population. Animals included in the sample are examined for the presence of disease and their status with regard to the presence or absence of specified risk factors. Various random sampling strategies can be used to select the study group, including stratified, cluster and multistage sampling. Sometimes it is referred to as frequency survey or prevalence study.

Usefulness

i. To estimate prevalence of the disease
ii. To set priorities for disease control
iii. To generate hypotheses

Merits

i. Relatively quick
ii. Relatively easy to perform
iii. Relatively cheaper

iv. Adopted for chronic diseases

Demerits

i. Not useful in establishing etiology
ii. Risk factors associated with disease risk cannot be distinguished from those linked with disease duration
iii. Not suitable for acute, short and rare diseases
iv. Not suitable for measuring incidence.

ii. Case-control

The case-control study compares diseased animals (cases) with non-diseased animals (controls) and therefore has variously been called a case-comparison, case-referent or case history study. This study selects groups according to presence or absence of disease and looks back to possible causes; it has therefore sometimes been described as a retrospective study. (looking back from effect to cause).

Usefulness

i. First step when searching for a cause of a health outcome
ii. Explore possible role of variety of exposures or characteristics in causing the disease

Merits

i. Less time consuming and less expensive than cohort study
ii. Useful in disease being investigated is rare
iii. Useful in diseases where time gap between exposure and development of disease is long.
iv. Small study size.

Demerits

i. Uncertainty of exposure-disease time relationship
ii. Inability to provide direct measure of risk
iii. How representative of all disease cases are the cases in the study?

iii. Cohort

A cohort study selects groups according to presence or absence of exposure to hypothesized causal factors, and then looks forward to the development of disease. It has therefore sometimes been called a prospective study (looking forward, from cause to effect).

Usefulness

i. If we have some idea of the exposures that are suspected as possible causes of a disease, cohort study can be conducted
ii. To find out if single exposure can cause many diseases
iii. Explore possible role of variety of exposures or characteristics in causing the disease
iv. Incidence can be estimated
v. Comparison of incidence between different subgroups can be analysed.

Merits

i. Direct determination of risk is possible
ii. Permit examination of multiple outcomes

Demerits

i. Long time to conduct

ii. Expensive
iii. Loss to follow up
iv. Exposures may change over time, leading to misclassification.
v. Difficult for rare diseases and long incubation period diseases

iv. Ecological study

In each of the above three types of study, it is necessary to know the exposure and disease status of all individuals. If this information is not available, characteristics of groups may then be studied, although an inference may still be required at the level of the individual. Such studies are ecological studies. Ecological studies should be interpreted with caution, but are useful preliminary indicators to causal hypotheses that should be tested more thoroughly with subsequent studies.

III. Experimental study design

An experimental study involves manipulation of the conditions of study (i.e. application of an intervention), and can be conducted either in a controlled environment, such as a laboratory, or in the natural environment of the animals (= field).

i. Experimental laboratory study

If it is conducted as an experimental laboratory study, great precision in measurement and optimal control of influencing variables can be achieved, resulting in sound cause – effect inferences. The disadvantage is that in a laboratory it is usually not possible to represent the myriad of factors affecting disease occurrence in an animal ' s natural environment, and it may be difficult to work with sufficient numbers of animals to represent true variation between animals in the natural population. Experimental studies typically involve dividing a group of animals into at least two subgroups: one to which an intervention will be applied and another so - called control or comparison group that will not receive the intervention. The decision to apply an intervention to a particular animal within the study or not should be based on random allocation (= randomisation). Such a study is also called a randomized controlled trial (RCT).

ii. Experimental field study

In contrast to the laboratory experiment, the animals will be exposed to all known and unknown factors present in their natural environment. These studies are usually used to evaluate therapeutic or preventive effects of particular interventions, but are also useful for investigating aetiological relationships. Experimental field studies are considered the method of choice for investigation of causal hypotheses about the effectiveness of preventive measures. Compared with observational studies they provide much better control over confounders and therefore can give stronger evidence about causality. The disadvantages of experimental field studies are that they often require large groups, they can be costly, the required duration can be long if disease incidence is low, and selection bias may be introduced if they are not designed appropriately.

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Measures of a Disease Occurrence

Dr. Jagadish Hiremath
ICAR – National Institute of Veterinary Epidemiology and Disease Inforamtics
Yelahanka, Bengaluru, Karnataka 560064

The objectives of veterinary epidemiology are to determine the extent of disease in a target animal population, identify patterns and trends in disease occurrence, identify the causes of disease, and evaluate the effectiveness of prevention and treatment activities. Measuring how often a disease occurs in a population is usually the first step in achieving these goals. Hence it is important to quantify the disease frequency in a population. The article will give an insight into the measures of disease occurrence, definitions, formulae to calculate different measures and effects. Finally a brief study frame is provided facilitate the research activities towards estimation of disease frequency.

Why to measure disease occurrence? (Fig.1)

- Measuring the amount of a disease in a population is essential to allow planning of animal health services *(Screening programs, building veterinary hospitals and diagnostic centers etc)*
- Measurement of frequency of disease helps to assess the extent to which new risk factors or preventive strategies influence patterns of disease in the population *(Import of animal and animal products, Disease control programs)*
- Measurement of patterns or the distribution of disease can identify disease clusters where further action/intervention and investigation may be warrant (Fig.1)

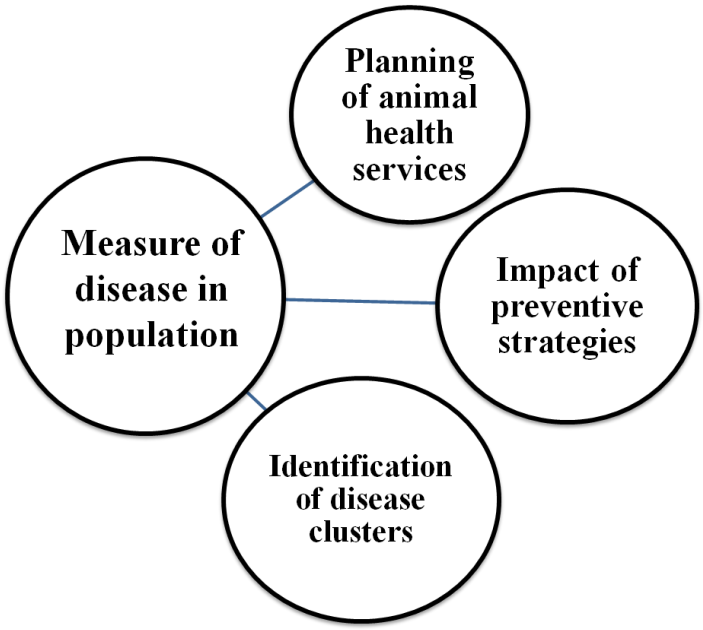

Figure 1 Use of measuring the disease in target population

Epidemiological measurements include Types of calculations, Measures of disease frequency and Measures of disease association (or Measures of Effects) (Table.1).

Table.1 Epidemiological measurement of disease in population

Types of calculations	Measures of disease frequency	Measures of disease association
Ratio	-	Risk Ratio
Proportion	Prevalence	Odds ratio
Rate	Incidence	Rate Ratio

Types of calculations

Three types of calculations are routinely used to calculate and compare the disease frequency *viz* ratio, proportion and rate (Fig.2).

Ratio: A fraction in which the numerator is not a part of the denominator and it is just one number divided by another. Ratio does not need numerator and denominator to be related. *Eg. For every 100 HS cases there are 40 FMD cases. Hence the ratio of HS: FMD is 5:2*

Proportion: A fraction in which the numerator is part of the denominator. *Eg. 100 cattle found positive for Brucellosis in a village with a total cattle population of 1800. Hence the proportion of cattle that are positive for brucellosis is 1:18*

Rate: A proportion over a particular period of time. An epidemiologic rate will contain the disease frequency (numerator), unit of population size, and the time period during which the event occurred.

Ex: *10 cases of Anthrax per 1,000 population in Kolar district of Karnataka during the year 2000.*

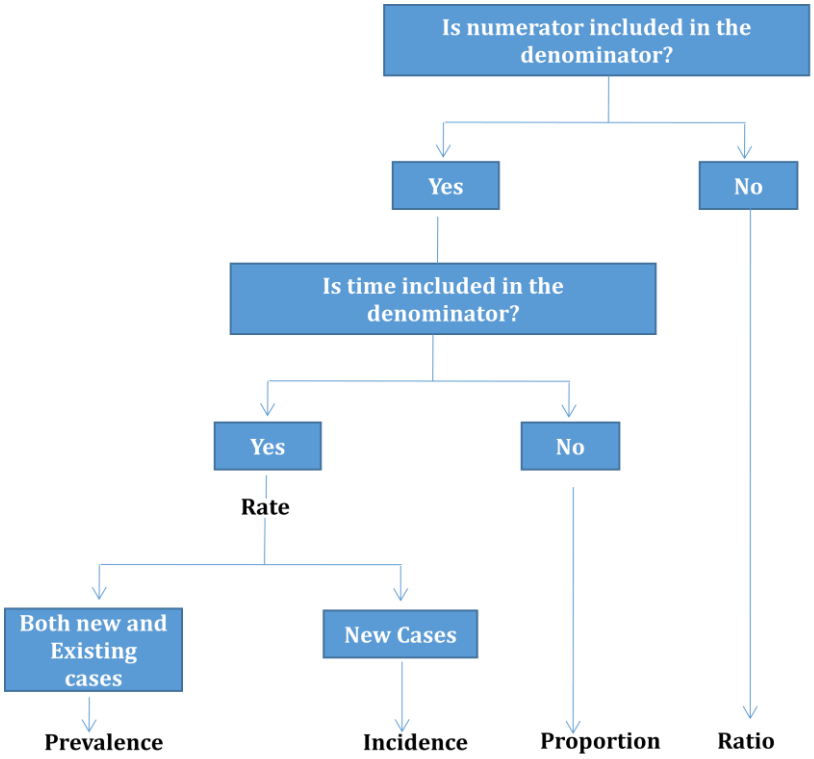

Figure 2 Distinguishing rate, prevalence, incidence, proportion and ratio

Measures of Disease Frequency

Prevalence: Prevalence measures the proportion of individuals in a defined population that have a disease or other health outcomes of interest at a specified point in time (point prevalence) or during a specified period of time (period prevalence). It is difficult to define a prevalent anthrax case. It is generally assumed that once diagnosed with anthrax, an animal/an individual represent a prevalent case until death.

However, this assumption is not always correct, as animals/people diagnosed with anthrax may survive for a long period with/without any recurrence of the disease and may die from another cause.

Note: Though prevalence, as a proportion does not have time unit, the point of time has to be specified as it may vary due to changes in disease status or migration of people over time.

$$Prevalence = \frac{Number\ of\ cases\ in\ a\ defined\ population}{Total\ animals\ in\ the\ defined\ population}$$

Point prevalence refers to the prevalence of a disease at a given point of time.

For example, in a city with 50 Anthrax cases in Cattle out of 250 total number of cattle at risk, the prevalence of disease will be 50/250, i.e. 0.2 or 20%.

Incidence: The number of cases of a condition present in a population at a point in time depends not only on the frequency with which new cases occur and are identified, but also on the average duration of the condition (i.e., time to either recovery or death). As a consequence, prevalence may vary from one population to another solely because of variations in duration of the condition. Measurements of incidence quantify the number of new cases of disease that develop in a population of individuals at risk during a specified time interval. Three distinct measures of incidence may be calculated: risk, odds of disease, and incidence rate.

$$Incidence = \frac{Number\ of\ animals\ affected\ during\ a\ certain\ period}{Number\ of\ animals\ at\ risk\ at\ the\ beginning\ of\ the\ time\ \ period}$$

Incidence rates (density) can be measured in a closed cohort or in an open population.

The relationship between prevalence and incidence:

The proportion of the population that has a disease at a point in time (prevalence) and the rate of occurrence of new disease during a period of time (incidence) are closely related.

Prevalence depends on:

1. The incidence rate (r)
2. The duration of disease (T)

For example, if the incidence of a disease is low but the duration of disease (i.e. until recovery or death) is long, the prevalence will be high relative to the incidence. For example diseases like Brucellosis or tuberculosis tend to persist for a longer duration, from months to years or some time life time; hence the prevalence (old and new cases) would be longer than the incidence.

Conversely, if the incidence of a disease is high and the duration of the disease is short, the prevalence will be low relative to the incidence.

A change in the duration of a disease, for example the development of a new treatment which

prevents death but does not result in a cure will lead to an increase in prevalence. Fatal diseases or diseases from which a rapid recovery is common have a low prevalence, whereas diseases with a low incidence may have a high prevalence if they are incurable but rarely fatal and have a long duration.

The relationship between incidence and prevalence can be expressed as:

P = ID

P = Prevalence
I = Incidence Rate
D = Average duration of the disease

A population in which the numbers of animals with and without the disease remain stable is known as a steady-state population. In such (theoretical) circumstances, the point prevalence of disease is approximately equal to the product of the incidence rate and the mean duration of disease (i.e. length of time from diagnosis to recovery or death), providing that prevalence is less than about 0.11.
That is Prevalence = Incidence x Duration

Measures of Disease Association:

Measures of effect are used in epidemiological studies to assess the strength of an association between a putative risk factor and the subsequent occurrence of disease. This requires that the incidence of disease in a group of animals exposed to a potential risk factor is compared with the incidence in a group of animals not exposed to the potential risk factor. This comparison can be summarized by calculating either the ratio of measures of disease frequency for the two groups or the difference between the two, and reflects the increase in frequency of disease in one population compared with another, treated as baseline.

Risk ratio: The relative risk (or risk ratio) is an intuitive way to compare the risks for the two groups.

$$Risk\ ratio = \frac{Cumulative\ incidence\ in\ the\ exposed\ group}{Cumulative\ incidence\ in\ the\ unexposed\ group}$$

Rate ratio: Rate ratios are closely related to risk ratios, but they are computed as the ratio of the incidence rate in an exposed group divided by the incidence rate in an unexposed (or less exposed) comparison group.

$$Rate\ ratio = \frac{Incidence\ rate\ of\ exposed\ group}{Incidence\ rate\ of\ unexposed\ group}$$

Table.2 2X2 Contingency table for estimating exposure risk

	Cases	**Control**	**Total**
Exposed	a	b	**a+b**
Unexposed	c	d	**c+d**
Total	**a+c**	**b+ d**	**a+b+c+d**

Odds Ratio (OR): The **Odds Ratio** is a measure of association which compares the odds of disease of those exposed to the odds of disease those unexposed. Appropriate formula to analyse associations between groups from case-control and prevalent (or cross-sectional) data (Table.2)

$$Odds\ ratio = \frac{aXd}{bXc}$$

FRAMEWORK FOR ESTIMATING SEROPREVALENCE OF A DISEASE

Estimation of seroprevalence is the most common epidemiological exercise done in the field. Usually it is the first step towards understanding epidemiology of disease. Unless a systematic epidemiological study approaches are made the estimates of seroprevalence will carry no meaning. Hence, the steps involved in estimating seroprevalence are shown below.

Study Population: It is essential to define the study population at the beginning of the epidemiological study (estimation of seroprevalence of Brucellosis) as the measure of disease estimates are population specific. The definition should clearly mention the inclusive and exclusive criteria of the study population.

Study Area: The geographical area taken for the study has to be defined as spatial factors differ from place to place and hence the disease level.

Time period: Time period is the time within which the disease study is carried out. This plays a crucial role in estimating seroprevalence of a disease in a place, at a given time. As diseases do not occur constantly through time, measures of occurrence are not possible without the period during which the population was at risk or the cases recorded. Time period also gives information whether the occurrence of a disease has increased or decreased over a period of time.

Study design: Different study designs provide information of different quality**.** Therefore it is important to determine the right study design. The cross-sectional study design is most commonly used study design for estimating seroprevalence of disease where in the data collected from a population, or a representative subset, is done at a specific point in time—that is, cross-sectional data

Case and Case definition: It is essential to develop case definition before deciding what a case is. The case definition lists the clinical criteria by which an epidemiologist can determine whether a person's illness can be considered as a case in an outbreak investigation, in the absence of an outbreak, a case definition is used in the surveillance of public health in order to categorize those conditions present in a population, such as Prevalence and Incidence of the disease. Case definitions are often used to label animals as suspect, probable, or confirmed cases.

Sampling techniques: To be able to generalize results from a sample to a population, a probability-based sample must be taken. Common sampling techniques include: Simple random sampling, systematic random sampling, and stratified random sampling.

Sampling size estimation: Sample size calculation plays an important role in epidemiological studies to achieve a desired precision in estimates of parameter of interest. Sample size is

calculated based on the type of Epidemiological study. Descriptive, observational and randomized controlled studies have different formulas to calculate sample size. Adequate sample size is required to ensure that the study will yield reliable information, regardless of whether the ultimate data suggest a clinically important difference between the treatments being studied, or the study is intended to measure the accuracy of a diagnostic test or the incidence of a disease.

Questionnaire design: Questionnaire can be of 3 types- Open ended questionnaire, Fill in the blanks type and Close ended questions (with categorical answers/ordinal answers). Close ended questions are chosen to estimate seroprevalence of a disease. Based on the required data, variables are decided and questionnaire is prepared for the data collection. Questionnaire is designed using software such as Epi Info or just typed out to collect information from paper. Variables are decided based on the statistical analysis to be used in the study.

Data collection, Visit to the field: Once the questionnaire is designed and tested, it is used for data collection. It is either using software installed in mobile/tab or using paper and pencil method. All equipments required for data/sample collection are carried to the field. Prior to data collection all field staff should be trained, all questions must be thoroughly discussed and a field manual on standardization of data collection procedures has to be produced.

Statistical Analysis: Data should be analyzed in order to estimate Prevalence of the disease at animal level/ herd level. Different statistical models are decided based on the type of study variables. Some of the common statistical models used are- Linear Regression, Logistic Regression, Polynomial model, stepwise regression etc. Prevalence of the disease can be calculated as shown before, using the ratio of number of positive animals to the population. Logistic regression model is used to identify risk factors of a disease and to quantify the status of a disease.

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A Practical Approach to Calculate Herd and Animal Level Sample Size

Dr. K.P.Suresh, Dr. S.S Patil and Dr. D Hemadri

ICAR – National Institute of Veterinary Epidemiology and Disease Inforamtics
Yelahanka, Bengaluru, Karnataka 560064

Introduction: In a large population where animals are separated into herds, disease has a strong tendency to cluster. This is because the disease agent or agents (whether infectious, environmental or genetic) are generally not evenly distributed throughout the population (Rothman, 1990). With rare diseases, this clustering is usually even more pronounced. As a result, a very low proportion of herds may be affected by a particular disease-but within those affected herds, the prevalence of the disease amongst animals may be quite high. If a survey designed to detect the presence of disease fails to take into account the clustering of disease in the population, the results of the survey are likely to be very unreliable. This is because the probability formulae that the surveys are based on assume that every unit in the population has the same probability of being affected. Another problem with large-area surveys is the logistics of sampling. Probability formulae assume simple random sampling. Simple random sampling of individual animals from a national herd requires the creation of a sampling frame which may need to list millions of animals (each uniquely identified). Such a sampling frame is usually impossible to construct.

The solution to both these problems is to use a two-stage sampling strategy in which herds form the first stage, and individual animals within selected herds, the second stage. In this way, the sample sizes at each stage can be adjusted to reflect the different disease prevalence's (the proportion of herds affected in the first stage, and the proportion of animals affected in the herd at the second stage). Two-stage sampling also means that the construction of sampling frames is much simpler. At the first stage, only a list of all herds in the population is required, and at the second stage, only animals in each of the selected herds is to be included in the list. However, the use of two-stage sampling presents particular problems for sample-size calculation and analysis.

The use of two-stage sampling has evolved to meet surveillance objectives for two reasons. First, list of frames of animals for randomized sample selection do not typically exist at a regional or national level, but the list frames of herds can be developed and maintained more readily. Secondly, the theory and applications of within-herd sampling with imperfect diagnostic tests is well developed. The within-herd sampling research has guided the approach to sampling to classify the herd`s disease or infection status.

The herd-level sensitivity (HSe or HSESNS) and Specificity (HSp or HSEPC) depend on the individual animal test characteristics, sample size, within herd prevalence etc., HSe and HSp are test characteristics which can be applied at the herd level in a manner equivalent to animal-level Sensitivity (Se) and Specificity(Sp) at the within -herd level. HSe and HSp usually are based on detecting infection if it is present above a fixed level, which is the level that is determined according to the epidemiology of the disease or specific national or international rules.

Methodology

The sample size calculated this way takes into account the sensitivity (Se) of the diagnostic method (the lower the Se the larger the sample size). Test specificity is not considered in this calculation. The lack of Sp of a diagnostic test produces false positive results

and increases the probability of a Type II error (that is, considering a population as affected by an event when it is actually free of it).

1. First stage Sample size Determination: Herd size

The sample size required for detection of an event if it is present in a population from two-stages sampling is determined by calculating independently the number of herds from which the individuals will be sampled and the number of individuals per herd to include in the sample.

Method 1: Based on the assumption of perfect classifications of each herd tested and of each animal, as either positive or negative. First stage sample size might be estimated using the simple formula based on the normal approximation to the binomial distribution (Snedecor and Cochrane, 1989)

HN=$\left(\frac{Z^2}{L}\right)$ HTP $(1 - HTP)$, where HN is the sample size (number of herds tested), HTP is the estimated Herd prevalence and L (usually 5 to 10%) is Tolerance around prevalence for the varying level of confidence Z (90%CI, 95%CI or 99%CI). This is based on assumption of approximately infinite population and perfect test.

Method 2: Survey design allowing for imperfections in the test.

This formula is based on Herd level sensitivity and Herd level specificity and also the assumptions of normal approximation to binomial distribution.

$$HN=\left(\frac{Z}{L}\right)^2 \left[\frac{(HSENS(HTP)+(1-HSPEC)(1-HTP))X(1-HSENS(HTP)-(1-HSPEC)(1-HTP))}{(HESENS+HSPEC-1)^2}\right]$$

where HSENS and HSEPC are the herd level sensitivity and specificity of test, HTP is herd level prevalence. L (usually 5 to 10%) is Tolerance around prevalence for the varying level of confidence Z (90%CI, 95%CI or 99%CI).

2. Second-stage sample size determination: Number of animals per herd

$$n_i = \left[1-(1-CL)^{\frac{1}{e}}\right] \times \left(N_i - \frac{e-1}{2}\right)$$

CL : The level of confidence, The confidence that the user wants to have in the results. Acceptable values: 90%, 95% or 99%.

e :The number of detectable individuals with the event in the population. This value is the Product of population size (N) by detectable prevalence. Detectable prevalence is the result of the product of expected prevalence (p) by Sensitivity (Se) of the diagnostic device or method

e=N x p x Se

Ni : Number of animals per herd

Valid estimates of herd-level prevalence can be obtained from population surveys using cluster sampling. Herds are selected at random and a diagnostic test is applied to randomly selected animals from these selected herds. Based on the results of individual-animal tests, each herd is assessed as either positive or not positive (thus providing a herd-level test so that the herd-level prevalence can be estimated). The difficulty with this approach lies in that most tests have imperfect animal-level sensitivity (HSENS) and specificity (HSPEC), which means that the categorisation of the herd as either positive or negative (i.e. herd tests) is also imperfect.

Results

Sample size for 2-stage survey with fixed herd/flock sensitivity can be calculated using the web based calculator " **http://epitools.ausvet.com.au/content.php?page=2StageFreedomSS_2**"

Calculate sample sizes for 2-stage surveys for demonstrating disease freedom, for specified target herd/flock sensitivity and system sensitivity. This analysis calculates the number of herds and the number of animals within each herd to be tested to provide specified herd and system sensitivities (probability of detecting disease) for the given animal and herd-level design prevalence and test sensitivity. Test specificity is assumed to be 100% (or follow-up testing of any positive will be undertaken to confirm or exclude disease).

Numbers of herds to test are calculated using the hyper geometric approximation if the number of herds in the population is specified as well as using the binomial formula assuming unknown (large) number of herds in the population. If the population size is not specified only the binomial results are presented.

Numbers of animals to test in each herd are calculated for a range of herd sizes using the hyper geometric approximation and for unknown (large) herd sizes using the binomial calculation.

Design prevalence (specified level of disease to be detected) must be specified at both animal and herd levels. Design prevalence can be specified as either:

- a proportion of the population infected; or
- a specific (integer) number of herds infected (for herd-prevalence only and only if the number of herds in the population is specified).

Inputs required include:

- animal-level design prevalence (as a proportion only);
- herd-level design prevalence and whether this is specified as a proportion or number of herds;
- the estimated test sensitivity;
- the target herd or flock sensitivity (SeH or HSENS) which is the probability of detecting disease if it is present in a herd at the specified animal-level design prevalence;
- the target system sensitivity (SSe) which is the probability of detecting disease if it is present in the population at the specified animal and herd level design prevalence's;
- The number of herds in the population (optional).

Outputs from the analysis include:

- The total numbers of herds to be sampled;
- The maximum total sample size (if animal-level prevalence is specified as a proportion);
- The numbers of animals to test in herd, for a range of herd sizes to achieve the specified value for SeH; and
- The numbers of animals to test in herd and the corresponding numbers of herds to test, for

a range of herd sizes and SeH values.

Hypothetical Example: For Animal level design prevalence of 10% , herd level design prevalence of 20%, Test sensitivity of 90%, Target herd sensitivity of 50% and target system sensitivity of 95% confidence interval, the results are presented as follows

Inputs

Animal-level design prevalence	10%
Herd-level design prevalence	20%
Test sensitivity	0.9
Test specificity	1
Target herd/flock sensitivity (SeH)	0.5
Target system sensitivity (SSe)	0.95
No. herds in population	Unknown

Number of herds to be sampled
mber of herds to be sampled

	Number of herds to sample	Maximum number of samples
No. herds in population unknown	29	232

Numbers of animals to be sampled for different herd sizes for SeH = 0.5

	Number of animals to sample
Herd size = 10	6
Herd size = 20	7
Herd size = 30	7
Herd size = 40	8
Herd size = 50	8
Herd size = 100	8
Herd size = 200	8
Herd size = 500	8
Herd size = 1000	8
Herd size = 5000	8
Herd size = 10000	8
Herd size = unknown	8

Numbers of animals to be sampled for different herd sizes and varying SeH

	SeH = 0.1	SeH = 0.2	SeH = 0.3	SeH = 0.4	SeH = 0.5	SeH = 0.6	SeH = 0.65	SeH = 0.7	SeH = 0.75	SeH = 0.8	SeH = 0.85	SeH = 0.9	SeH = 0.95
Herd size = 10	2	3	4	5	6	7	8	8	9	9	10	10	
Herd size = 20	2	3	4	6	7	9	10	11	12	13	14	16	18
Herd size = 30	2	3	4	6	7	9	10	12	13	14	16	18	22
Herd size = 40	2	3	4	6	8	10	11	12	14	15	17	20	24
Herd size = 50	2	3	4	6	8	10	11	12	14	16	18	21	26
Herd size =	2	3	4	6	8	10	12	13	15	17	20	23	29

100													
Herd size = 200	2	3	4	6	8	10	12	13	15	18	21	25	31
Herd size = 500	2	3	4	6	8	11	12	14	16	18	21	26	33
Herd size = 1000	2	3	4	6	8	11	12	14	16	18	21	26	33
Herd size = 5000	2	3	4	6	8	11	12	14	16	18	22	26	34
Herd size = 10000	2	3	4	6	8	11	12	14	16	18	22	26	34
Herdsize = unknown	2	3	4	6	8	10	12	13	15	18	21	25	32

Numbers of herds to be sampled for varying SeH

	SeH = 0.1	**SeH = 0.2**	**SeH = 0.3**	**SeH = 0.4**	**SeH = 0.5**	**SeH = 0.6**	**SeH = 0.65**	**SeH = 0.7**	**SeH = 0.75**	**SeH = 0.8**	**SeH = 0.85**	**SeH = 0.9**	**SeH = 0.95**
No. herds in population unknown	149	74	49	36	29	24	22	20	19	18	17	16	15

Conclusion:

The herd-level sensitivity and specificity needs to be decided by the investigator. For a given screening test, the herd-level sensitivity and specificity can be set to a range of values by varying the sample size. While sensitivity plays a role in the sample size, herd-level specificity is more important, due to the much greater proportion of false positive results than false negative when prevalence is very low. If specificity is increased, the number of herds that needs to be sampled decreases, but the number of animals that must be sampled from each herd to achieve this level of herd test specificity increases. The choices of herd-level sensitivity and (especially) specificity therefore determine the balance between the required number of herds and the number of animals per herd.

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Sample Collection, Packing and Transportation

Dr. P. Krishnamoorthy

Scientist

ICAR – National Institute of Veterinary Epidemiology and Disease Inforamtics
Yelahanka, Bengaluru, Karnataka 560064

Sample

The term "sample" may either mean a specimen (animal, blood sample and others) or, used in the statistical sense, a sub-collection or sub-set of units. The aim in general is collection of samples representative of the study population. It is difficult to collect materials required for disease diagnosis from all the animals in a population. Hence the samples are collected to represent the population; usually samples are collected at random. Clinical samples means samples collected from an ailing or affected animals with particular diseases.

Collection of samples

Sample collection is done mainly for direct examination of the sample from the animals, isolation of microorganisms for definitive diagnosis and serological diagnosis which will help in the diagnosis of animal diseases.

Collection of specimens for culture differs from collection of specimens for routine analysis.

1. Avoiding contamination by the organisms on the skin is essential if misleading results and inappropriate therapy are to be avoided.
2. Although any small volume can be cultured, the probability of obtaining a "positive" culture increases in proportion to the size of the sample obtained. Sub-optimal samples can result in the inappropriate diagnosis of disease.
3. Collect specimens in sturdy, sterile, screw-cap, leak proof containers with lids that do not create an aerosol when opened.

Aerobic Culture Swab

The use of the aerobic culture swab is recommended for most body sites for which a swab of such size is a suitable method to collect the specimen.

1. Collect the specimen and replace the swab in the plastic container.
2. If the collection swab has an ampoule, crush it to ensure that the transport fluid comes into contact with the swab. If the collection swab has a moistened sponge, no action is needed.
3. Label with animal number, age, sex, breed, body site, type of sample and date and time of collection.
4. Refrigerate specimen for transport. Deliver to lab within 48 hours.

Anaerobic Culture Collection

1. The sample should be collected from the active site of infection and precautions should be taken to exclude surface contamination and the aeration of the sample. Whenever possible, specimens should be pus or fluid obtained by needle aspiration through intact skin or mucosa, which has been cleansed carefully with antiseptic.
2. In situations where material must be obtained from open foci of infection, sinus tracts or drainage tracts, it is best to aspirate purulent material with a syringe attached a sterile plastic

catheter. The assembly can be passed deeply into the sinus tract or wound after the surface opening has been mechanically cleaned with a non-germicidal agent.
3. If irrigation is required to obtain an adequate specimen, lactated Ringer's or non-bacterostatic normal saline (sterile) may be used. Broth should not be used.
4. Swabs may be used only as a last resort and they must be stored in an oxygen-free atmosphere. As much specimen as possible must be taken up on the swab so that the tip is saturated. A second swab is used for the Gram Stain.
6. Tissue suspected of containing anaerobes should be placed in appropriate anaerobic containers.

Collection of blood and serum

Before collection of blood, make the area of skin surface sterile by using alcohol swabs. Collect blood from animals using sterile vacutainer tubes if blood is used for isolation of microorganisms. The vacutainer needles are very useful in collecting blood with less pain to animals. The blood may be collected in the tube containing Dipotassium EDTA coated or heparin coated tubes. Collect 5 to 8 ml of blood per animal which can be used for various tests and diagnosis of diseases. For serum collect the blood in tube coated with sodium silicate vacutainer tubes which favours clot formation and easy separation of serum. The serum should be separated by centrifugation at 2000 rpm for 15 to 20 minutes. The serum collected may be used for the serological investigations and it should be stored at -20ºC until used. Blood can be used for isolation of brucella organisms and also to extract DNA to confirm by PCR. Serum can be used for detection of antibodies against brucella organisms.

Filter paper technique

Cut the filter paper into strips of size 4 cm x 1cm and pin at the centre by using three strips. It makes the six ends for collecting the blood sample. Put few drops of blood on the filter paper strips and allow it to dry. Then the filter paper can be sent by post to the concerned laboratory for diagnosis of animal diseases. The dried blood can be used for isolation of DNA and confirmation of disease suspected by PCR may be done. This technique is an easy one and involves less transportation and storage requirements.

Tissue collection

The tissues samples are usually collected from the dead animals for identifying the cause of the death. Tissue samples immediately after collection it should be fixed in proper fixative for histological examination. Usually, 10 per cent formalin is used and it is prepared by adding 10ml of 40 per cent formaldehyde with 90ml of tap water to make 100ml of 10 per cent formalin. In carcasses of rare species, preservation of organs *in situ* for anatomical studies may be useful, with only small samples taken out for examination, causing as little disturbance as possible. If a necropsy is only done for detection of health problems, enough tissue for examinations including bacteriology should be taken, samples including abnormal areas and surrounding normal areas. It is recommended samples no thicker than 1 cm (for good fixation), but long and wide enough to represent different areas of a tissue and possible abnormalities to be collected. In small animals, entire organs instead of samples may be collected. The mechanical damaging of samples by compressing them with forceps may be avoided.

Collection of nasal swabs

The nasal swabs can be collected from the posterior nasal passages using a sterile swabs moistened with transport medium or saline or phosphate buffer saline. The swabs should be collected by using readily available sterile swabs in plastic tubes and after collection it can be placed immediately in the culture tubes containing culture media for bacterial isolation purposes.

Collection of faecal swabs

The faecal swabs are collected for bacterial isolation or for determination of parasites in the faeces. The moistened swabs are inserted into the rectum and rotated clockwise and anticlockwise, collect the faeces and immediately put in the culture tubes for isolation of microorganisms.

Collection of skin scrapings

The skin scrapings can be collected using sterile scalpel and foreceps and collected in the paper. It is collected mainly for examination of external parasites.

Collection of cerebrospinal fluid

The lumbo-sacral joint between the last lumbar vertebrae and the anterior end of sacrum is preferred site for collection of cerebrospinal fluid. The skin surface is shaved, washed with detergent and disinfected with alcohol. The sample is collected using the sterile spinal needle or 18 gauge needles with aspiration by 10 ml syringes.

Procedures to be following during transport of clinical samples

1. Do not transport material for culture in the needle and syringe. Needle transport is very unsafe because there is always the risk of a needle stick injury, and syringe transport poses a risk because specimen may be expelled during transport, creating a threat to personnel and environment. Transfer aspirated material to a tight container.
2. Place tissue samples, biopsy samples into an anaerobic transport device or a sterile tube or petri dish. Place all of this into a sealable plastic bag that generates an anaerobic atmosphere which will prevent contamination.
3. The sample containers should be properly labeled and identified. The labels like “Clinical Specimen, Fragile and Handle with care” may be written on the container or the parcel containing the clinical samples.
4. The sample container should have the details like nature of the clinical sample, animal number, place and date of collection which is important for easy identification and tracking of samples.
5. The samples should be sent with proper sample details and history of the case in a separate sheet which will help in the appropriate tests to be carried out for particular diseases.
6. Blood smears should be dispatched after fixing in methyl alcohol or suitable fixatives.
7. The serum samples should be transported on ice at 4º C to prevent decomposition of the serum proteins.
8. The tissues samples should be transported in transport media or directly on ice as early as possible to prevent autolysis of the tissues.
9. Milk samples should be dispatched as quickly as possible, if dispatch is delayed then add 1 part of 5 per cent boric acid to 10 parts of milk as a preservative.

10. For isolation of virus, the specimens collected should be stored in 50 per cent glycerol saline or phosphate buffer solution containing 5 per cent bovine serum albumin and antibiotics.

Storage of clinical samples

1. The samples are stored if the processing is delayed and usually done using refrigeration.
2. The blood samples should be processed immediately but can be stored for 24 to 48 hours in refrigeration at 4º C.
3. The serum can be stored at freezing conditions at 0º C or -20º C for long time storage without decomposition of the serum proteins.
4. The tissues should be stored at -40 to -80º C if it is to be stored for long period.
5. All the clinical samples should be chilled to refrigeration immediately after collection if the processing is delayed.

Bacterial diseases:

Name of the disease	***Symptoms of the disease***	***Clinical specimens of choice for laboratory diagnosis***
Anthrax	A zoonotic disease caused by *Bacillus anthracis,* is characterized by high fever, bloat, respiratory distress due to oedema of thorax and brisket region, muscular tremors, abdominal pain and sudden death followed by bloody discharges from natural orifices.	1. Blood smears from ear vein, swelling and discharges from natural orifices, peripheral blood, heart blood, spleen and swollen lymph nodes for demonstration of bacilli. 2. Ear tip or a piece of muzzle in saline for isolation of anthrax bacilli.
Brucellosis	A zoonotic disease causing contagious abortion and infertility. It is caused by *Brucella abortus* in cattle, *B. melitensis* in sheep and goats, *B. suis* in pigs.	1. Milk, for milk ring test and isolation. 2. Serum sample (paired serum sample) for serological tests. 3. Vaginal mucus, uterine fluid and blood on ice for isolation and PCR. 4. Semen samples and swabs from the male reproductive organs for isolation and PCR. 5. Stomach contents of aborted foetus, on ice for isolation or PCR.
Campylobacter infection	Contagious venereal disease of cattle characterized by abortion, infertility with repeat breeding caused by *Campylobacter foetus*	Vaginal mucus swabs and preputial washing in sterile swabs and stomach content of aborted fetus on ice for isolation of Campylobacterium.
Black Quarter (BQ)	BQ is a disease of sheep and cattle and caused by *Clostridium chauvoei* bacteria. A symptom is characteristic swellings which	1. Impression smears from the affected muscle and exudates from the swelling for demonstration of Causative

	make a crackling sound under pressure.	organisms. 2. Pieces of affected muscle and intestines on ice for isolation of *Cl. chauvoei*
Enterotoxaemia (ET)	An infectious disease of ruminants caused by *Clostridium perfringens* and characterized by abdominal pain, hemorrhagic enteritis and sudden death. Symptoms vary depending upon the type of toxin produced by the organism (types A, B, C, D, E, F etc.).	1. Smears from contents of small intestine for demonstration of Gram positive rods with spores. 2. Contents and pieces of small intestine, blood on ice for isolation of Clostridium.
Haemorrhagic Septicaemia (HS)	Caused by *Pasteurella multocida* and the disease is characterized by high fever, localized oedema and respiratory symptoms.	1. Smears from peripheral blood, fluid from swelling, impression smear from heart, lungs, liver, submaxillary swellings for demonstration of bipolar organism. 2. Blood in sterile container for isolation. 3. Swabs from exudates, heart blood and pieces of liver, spleen and kidney, lymph nodes on ice for isolation of pasteurella.
Leptospirosis	A zoonotic disease caused by the different species of the Genus Leptospira. The disease is seen as an acute or chronic or clinically inapparent condition and is characterized by sudden fever, muscle tremors, anorexia, haemoglobinuria, icterus and abortion.	1. Blood and serum for dark field microscopic observation, isolation and PCR of leptospires. 2. Tissue from kidney, liver and spleen in 10% formalin for histopathology. 3. Milk or urine in vials (on ice) for isolation.
Listeriosis	Listeriosis or circling disease is a fatal infectious disease of man and animals caused by *Listeria monocytogenes*. The disease is characterized by encephalitis, abortion or septicemia.	1. Blood, cerebrospinal fluid, medulla and portion of spinal cord, brain tissue, aborted foetus or placenta on ice for isolation of listeria. 2. All internal organs in 10% formalin for histopathology.

Johne's disease (paraTB)	A chronic, infectious, fatal gastrointestinal disease of ruminants caused by *Mycobacterium johnei.* The most cardinal symptom is continuous or intermittent diarrhea leading to progressive emaciation and death.	1. Rectal pinch swab or smear for demonstration of Johne's bacilli. 2. Faecal samples, terminal portion of ileum with ileocaecal valve on ice for isolation of acid fast organisms.
Bovine Tuberculosis (TB)	A chronic contagious disease of man and animals caused by different species of *Mycobacterium.* The disease is characterized by a painful, dry, hacking cough, respiratory distress, abdominal pain, diarrhea, chronic bloat, emaciation, irregular oestrus cycle, abortion, sterility, formation of small nodules in mammary tissues, painful swellings of the joints, etc.,	1. Sputum and nasal swabs and milk in and lymph glands or lung lesions in sterile container on ice for isolation. 2. Heat fixed impression smears from bronchial lymph glands for staining. 3. Affected tissue like lungs in 10% formalin for histopathology.
Glanders	A zoonotic disease usually seen in horses caused by *Burkholderia mallei*. The disease is characterized by nasal discharge, formation of small nodules on upper respiratory tract mucosa and along the lymphatic channels of the skin and presence of ulcers on the skin.	1. Nasal discharge and pus from skin lesions on ice for isolation of bacteria. 2. Impression smears of pus for Grams staining. 3. Affected tissues in 10% formalin for histopathology.
Mastitis	Caused by different species of bacteria in cattle, buffalo, sheep, goats, pigs	Milk samples (mid-stream) before onset of treatment in sterile vials on ice.

Viral Diseases:

Disease	Symptoms of the disease	Clinical specimens of choice for laboratory diagnosis

Food and Mouth disease (FMD)	The disease is caused by picornavirus, the prototypic member of the genus *Aphthovirus* infecting cloven hoofed animals and is characterized by high fever that declines rapidly after two or three days, blisters inside the mouth that lead to excessive secretion of stringy or foamy saliva and to drooling and blisters on the feet that may rupture and cause lameness.	1. Vesicular epithelium or oeso-pharyngeal fluid in 50% Phosphate Buffered Glycerine for isolation of virus. 2. Sera sample for diagnosis.
Rabies	It is caused by Rhabdoviridae family which infect all warm blooded animals and highly zoonotic. The symptoms include slight or partial paralysis, cerebral dysfunction, anxiety, insomnia, confusion, agitation, abnormal behavior, paranoia, terror, hallucinations, progressing to delirium and death.	1. Head / whole carcass on ice for demonstration of viral antigen, viral inclusions and isolation of virus. 2. Brain on ice for demonstration of viral antigen, viral inclusions and isolation of virus. *Note:* It is not advisable to open the skull by persons not protected by vaccination
BlueTongue (BT)	It is caused by Orbivirus which infect sheep, goats, cattle and important signs are high fever, excessive salivation, swelling of the face and tongue and cyanosis of the tongue. Swelling of the lips and tongue gives the tongue its typical blue appearance.	1. Collect the blood in heparin or EDTA when body temperature is at its peak. Spleen, lung and lymph nodes on ice for isolation of virus. 2. Paired sera in sterile vials on ice for serological investigation. 3. Spleen, lymph nodes, intestine in 10% formalin for histopathology.
Infectious Bovine Rhinotracheitis (IBR)	Caused by BHV-1 and involved in several diseases worldwide and in cattle it causes rhinotracheitis, vaginitis, balanoposthitis, abortion, conjunctivitis, and enteritis.	1. Sera for detecting antibodies by serological tests. 2. Swabs from vaginal, conjunctival and nasal lesions and pieces of trachea and lungs in 50% Phosphate Buffered Glycerine on ice for virus isolation. 3. Pieces of trachea, liver, turbinate bone, lungs in 10% formalin for histopathology.

Sheep and goat pox and Orf	Caused by a Capripox and parapox viruses, respectively. Symptoms include papules and pustules on the lips and muzzle, and less commonly in the mouth of young lambs and on the eyelids, feet and teats of ewes. The lesions progress to thick crusts which may bleed.	1. Collect the blood at the height of body temperature in heparin or EDTA, scab and pustular materials, spleen, lung and lymph nodes on ice for virus isolation. 2. Paired sera in sterile vials on ice for serology. 3. Spleen, lymph nodes, intestine in 10% formalin for histopathology.
Peste des petitis ruminants	Caused by Morbillivirus. Infects goats and sheep and most typical signs are rise in body temperature, diarrhoea, ulceration of the buccal mucosae, especially on the inner face of the lips and neighboring gum, serous nasal exudates and conjunctivitis.	1. Citrated blood, eye, mouth and rectal swabs and pieces of spleen, lymph nodes, intestine in PBS on ice for isolation of virus. 2. Sera samples for serological tests. 3. Lungs, liver, spleen, tonsil in 10% formalin for histopathology.
Swine Fever (CSF)	Swine fever causes fever, skin lesions, convulsions particularly in young animals and death within 15 days.	1. Heparinised blood at the height of temperature for isolation, pieces of spleen, mesenteric lymph glands, intestine especially ileocaecal region in 50% glycerol saline for isolation of virus. 2. Pieces of brain, lung, intestines, ileocaecal region and kidney for histopathology.

Parasitic diseases:

1.	**Theileriosis**	Blood smears, biopsy smears from swollen lymph nodes from early stage of disease fixed with methanol
2.	**Babesiosis/ Anaplasmosis**	Thin blood smears fixed in methanol.
3.	**Surra/Trypanosomiasis**	Wet film examination of blood by hanging drop, fixed blood smears, blood in anticoagulant on ice.
4.	**Schistosomiasis**	Nasal schistosomiasis –Nasal discharge and nasal granuloma in normal saline.
5.	**Trichomoniasis**	Vaginal or uterine discharges, preputial scraping/ washing.
6.	**Gastro-Intestinal Parasitic Diseases**	Dung sample and affected internal organs in 10% formalin.

7.	**Ectoparasitic Infestations (Ringworm, Mange, Mites)**	Deep skin scrapings in sterile vials.
8.	**External Fungal Infections**	Skin scrapings in sterile vials

Thus the proper and systematic collection, dispatch and storage of samples are necessary and given at most care for proper diagnosis of animal diseases. The effort made in diagnosis will go waste if proper or representative samples are not collected. Hence the collection of samples should be given more importance during the diagnosis of animal diseases.

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Developing Effective Questionnaire for Epidemiological studies

Bhavana G. B and Dr. Jagadish Hiremath

ICAR – National Institute of Veterinary Epidemiology and Disease Inforamtics
Yelahanka, Bengaluru, Karnataka 560064

Introduction

Questionnaire is a measuring tool used frequently in observational studies to record the data. Since epidemiology is an observational science, where in the researcher do not have control over the variables, questionnaire is the only way to systematically collect the statistically analyzable data to answer the epidemiological question. The identification of disease risk factors, risk assessment, estimating vaccine effectiveness and vaccine coverage, etc., involves use of well design questionnaire as an effective instrument to collect the relevant data (Fig.1).

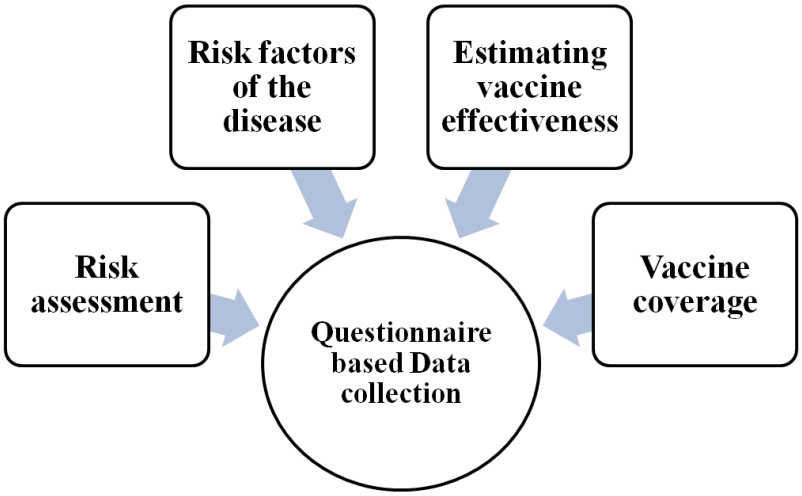

Fig.1 Use of questionnaire in epidemiological studies

In general, an epidemiological study aims at finding out the reason for disease occurrence in a given place, at a given time and in a given host. Towards this it is critical to give a definition of geographical area of the study, period of the study and affected population. Finally it is the questionnaire which will aid in collecting field data related to space, time and the target population (*Example1*)

Example1:

Study question: What are the risk factors of Brucellosis in Karnataka?

To answer this question it is essential to follow steps listed below

1. Define the study area
2. Define the study population
3. **Develop a questionnaire with following components**
   Factors associated with Host (Species, Breed, Age, Sexual maturity etc.,), Environment factors (Temperature, Rainfall, Husbandry practices etc.,)
   Farm Biosecurity Measures, Community awareness, Vaccination status

A questionnaire hence is a set of questions for obtaining statistically useful information. In most of the investigations, respondents are the only ones that can provide such detail information on the required variables. For example, a detail investigation involving Brucellosis outbreak can be done by taking responses from the owners of the animals affected rather than a veterinarian of the place/the veterinary hospital. Questionnaire can collect both quantitative and qualitative data depending on the requirement.

Designing Questionnaire

It is important that the questionnaire is designed such that it measures what is intended to measure. Composing and validating a questionnaire requires attention to its flow, format and length.

Efficient implementation of the questionnaire design process

1. Research Question: The first step towards designing a questionnaire is to know the research question.

Eg. 1. What are the risk factors of Brucellosis in Bovines in Punjab?

2. What is risk of getting PRRSV from north east India to rest of India?

3. What is the effectiveness of post-exposure prophylaxis anthrax vaccine in sheep/goat?

4. What is vaccine coverage for vaccination against Brucellosis in Gujarat?

5. What are the risk factors of Leptospirosis in Canines in Mumbai?

2. Literature review: Once the research questions is clearly framed the next step is to start the review of existing literature, other data sources including statistical and non statistical, surveys etc with similar research topics to identify the measurable parameters (Objective or subjective) relevant to answer the research question.

Eg. What is the effectiveness of post-exposure prophylaxis anthrax vaccine in sheep/goat?

Search for the papers to identify the factors to be included in questionnaire, like vaccine manufacturing companies, route of vaccine, vaccine dose, vaccination before exposure, vaccination after exposure, time of first vaccination, Breed of sheep/goat, Age etc.,

3. **Survey objectives specification**: Based on literature review frame the objectives and activities of the study. The objectives should be specified before construction of the questionnaire. Decision should be made on target population, data collection mode and sampling design. Besides informal communication with experts and users, expert group

meetings involving key users should be held in order to identify the concrete information demand to be satisfied.

4. Conceptualisation: Concepts have to be clear and translated into observable variables. These variables/indicators are suitable representatives of the concept. For example, if the concept under consideration is vaccine effectiveness, one possible measure is outbreak data since last outbreak, again this can further be subdivided into various sub parameters. A number of qualitative methods are available in order to get an idea of how respondents think about what survey designers have conceived.

Focus groups are composed of a small number of target population members guided by a moderator. The objective is to learn how potential respondents use the terms related to the topic, how they understand general concepts or specific terminology, and how they perceive the questions in terms of sensitivity or difficulty. Additionally, focus groups could also be a useful method for pre-field testing of questionnaires.

In-depth or qualitative interviews in a similar way focus on the respondents' understanding of the concepts, on how the respondents interpret certain questions and on how they arrive at their answers. In contrast to focus groups, in-depth interviews are not based on a group discussion.

5. Definition of a list of variables and a tabulation plan: Once the objectives and concepts are defined, a concrete tabulation plan and a list of variables should be laid down specifying the expected output of the survey. The variable and value list is to be seen as a simple list of variable names and values as well as of the corresponding definitions (Example 2)

Example 2: In the study mentioned in Example 1, different types of variables can be categorized as

Variable Type	Variable	Values
Categorical/ Qualitative	Status of Brucellosis	Yes/No
	Stage of pregnancy	I, II, III
Continuous/ Quantitative	Age of the cattle	Number of years
	Milk yield after vaccination	Number of litres/day

Above table shows the variable type and the kind of variables used in the questionnaire for the study of Brucella risk factors.

6. Data collection mode: The selection of an appropriate data collection mode must take into account the number, the contents and the scope of the data collection variables. It is important

that the questionnaire should not be designed without a previous decision on the data collection mode (Table 1)

Table 1: Different data collection modes and type of administration

Technology	Type of administration	
	Interviewer administration	***Self-administration***
Computer-assisted data collection	Computer-Assisted Personal Interviewing	Computer-Assisted Self-Interviewing
	Computer-Assisted Telephone Interviewing	Web Based Survey or E-Mail Survey
		Touch-tone Data Entry
Paper and Pencil	Paper and Pencil face to face interview	Paper and Pencil mail surveys

7. Writing and sequencing the questions: Questionnaires must translate the variables to be measured into a language that respondents understand. It is essential that the words used in questionnaires have the same meaning for all the respondents and at the same time correspond to the concepts to be measured. In order to retrieve necessary information from the respondent, the questionnaire should take of the following types of questions:

a) Questions should be framed using simple terms which have the same meaning for all members of the target population (Table 2, Q1)
b) The answer categories must cover all possible responses and correspond as closely as possible to the variation in the population (Table 2, Q7)
c) The respondents and/or interviewers should be provided with clear instructions. Instructions should however only be used if necessary and should be kept reasonably short.

d) The questionnaire structure should encourage respondents to answer the questions as accurately as possible. To this end, the questionnaire must focus on the topic under study, be as brief as possible, flow smoothly from one question to the next, facilitate respondents' recall and direct them to the appropriate information source.

e) The sequence of questions should follow a logical stream. It should flow from less complex to more complex questions and questions belonging to a common topic should be categorized and grouped. For example, all questions that refer to maintain biosafety should be grouped under the 'biosafety measures on the farm'

f) Order of questions also has an impact on the results. The respondent's interpretation of a certain question may be influenced by earlier questions. To facilitate the measurement of sensitive questions, the topic should be reached gradually and in a

way that makes the respondent feel at ease, and the wording should be as neutral as possible (Table 2, Q3)

Table 2: Problems faced with questions while designing a questionnaire

Sl. No.	Problem	Example	Issue	Solution
1	Unclear	"Have you ever fed commercially available food to your animals"	What does commercial food refer to? Naturally available food that is brought or processed food should be specified	"Do you feed processed commercially available food to your cattle"
2	Non self explanatory	"How heat is delivered to your home"	The investigator is interested in whether heat is delivered in form of hot air, water or steam. However question maybe answer as "heat was transferred in fuel truck"	All possible answers required are to be read/ written after the question, so that the respondent can select the relevant answer.
3	Two questions combined	"Have you ever vaccinated the animal"	Which disease vaccination does it refer to and when are to be mentioned	"When was the last time you vaccinated the animal for FMD" (After asking if they vaccinate their animals)
4	Difficult words to understand	"Is the animal gravid"	Many farmers wouldn't understand Words like 'gravidity', instead use Pregnant which is easily understood	"Is the animal Pregnant"
5	Events difficult to remember	"What was the date you sold your last cattle"	They might have sold them long back or haven't sold at all. Researchers shouldn't take it for granted that they'll remember dates of events from long back	Questions related to events that occurred long time back should be measured with caution
6	Hypothical questions	"Would you like to take your animals to the lake"	If time permits, most of them would take their animals	Certain situation correlate poorly with actual situation
7	Categories	"You visit the haat because of price/transport?"	The answer can be distance or something else as well	There has to be an option 'others' as well or all possible answers
8	Too long questions	"Have you ever administered antihelmenthic/antibiotic/ diuretic for your cattle"	The question is straight forward but it is too long. It is human nature to remember the last item only	This could be divided in different questions and medicines taken should be asked separately
9	Question details	"List out each brand of medicine being administered and since when"	Respondents are being asked too many questions at once	This should be asked in systematic series
10	Leading Questions	"Do you take your cattle for grazing regularly"	There are high chances that the respondent will answer yes	Instead you can ask, "Do you take your cattle for grazing at least 20 minutes everyday"

Electronic questionnaire design: Electronic questionnaires have same main objectives as the Paper and Pencil questionnaires. Computer assisted interviewing requires questions to be framed using a software. Collection of data is easy and the information is specific.

The questionnaire for paper and pencil technology can be designed as a word document or using software such as Epi info, designed for epidemiology data collection and analysis, by CDC (Fig. 2). This software can be downloaded to windows or for the mobile as well, which facilitates easy data collection. (Download link: https://www.cdc.gov/epiinfo/index.html)

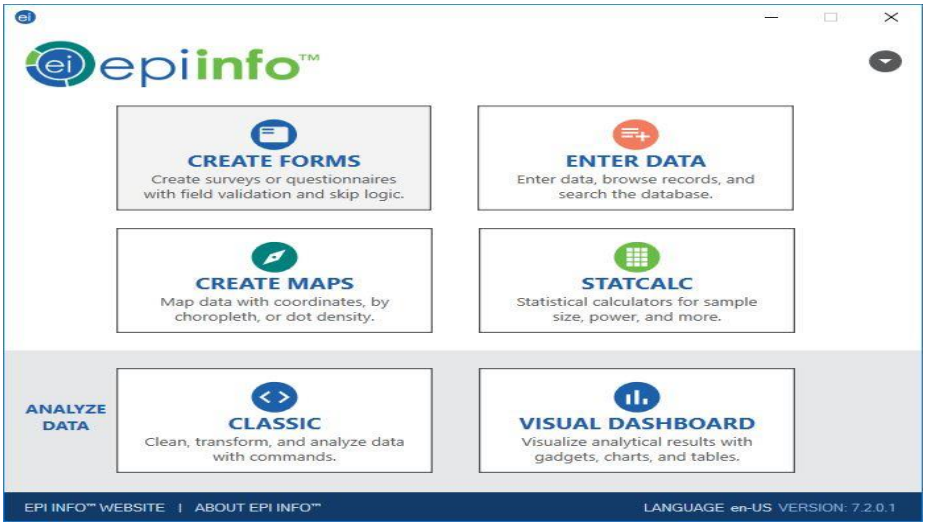

Fig.2: Epi-Info layout after the software is installed and opened

Points to be noted while designing questionnaire using software

i. Careful planning and addition of the questions is very essential.
ii. Some values require coding, which is understood by certain software for analysis. For example, a question that requires Yes or No as an answer Yes is coded as 1 and No as 0.
iii. Questionnaires should be easily adaptable to different hardware and software and be efficient in terms of response time for screen replacement.

Questionnaire testing: Questionnaire testing is critical for identifying problems for both respondents and interviewers with regard to, e.g. question wording and content, order/context effects, and visual design. Problems with question wording include, for example, confusion with the overall meaning of the question as well as misinterpretation of individual terms or concepts. There are two major categories of questionnaire testing methods- Pre-field testing and field testing.

Pre-field testing: It is normally applied under the laboratory conditions. Here, few respondents are selected and asked the questions. Further, based on the respondents' perception and answers, questions are finalized, modified or removed, such that the questions are more relevant and to the mark for subsequent field testing. They include expert group reviews and cognitive interviews such as think aloud interviews, probing, respondent debriefings, confidence ratings, paraphrasing, sorting, vignette techniques, and analyses of response latencies. The observations may differ from the actual field interview as this is carried out in a focus group of people, space and time.

Field testing: It refers to pre-analysis of the questionnaire on the field, which means that the interview is carried out exactly similar to the subsequent field interview and majority of the conditions are same as the actual environment of the interview. Test can be conducted during data collection phase, as a pilot study. Field testing always involves bigger sample sizes and allows quantitative analyses. The focus is more on the complete questionnaire instead of individual questions. Field methods include behaviour coding, interviewer debriefings, respondent debriefings, follow-up interviews, experiments.

Revision: Based on the responses from the respondents, questionnaire is revised and the review team makes changes accordingly before it is taken to the field for data collection.

Data collection: Based on the data collection mode, equipments required for data collection should be present in adequate quantity, for example in Paper and pencil type, there should be enough questionnaire copies available before leaving to the field. A GPS device is carried along in case there is requirement of the latitude and longitude of the places from where the samples are collected. This further helps in representing the places on a map. In case of data collection using Epi info created forms, Epi info android app can be downloaded in the mobile and carried to collect the data. This method is more useful, as it saves time for data entry and avoid confusions caused between data entry in the forms and feeding it into the software.

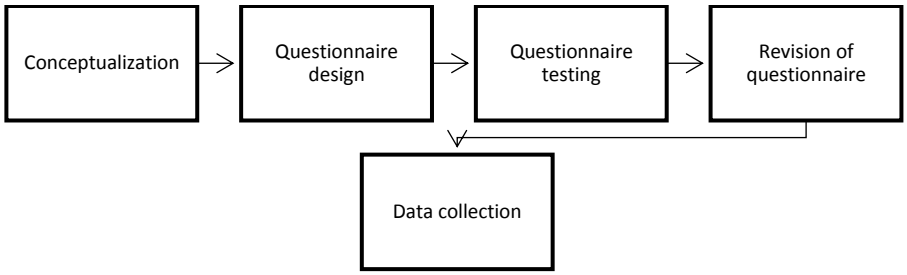

Figure 2: Stages of questionnaire design and testing

Epidemiological survey is an effective way to collect the observational data but if not carefully planned and designed they tend to associate with many types of error. The measuring error is contributed by improperly designed questionnaire. Hence a clear understanding of research questions and proper framing of questionnaire is must for successful epidemiological research.

Further reading:

1. European commission grant agreement - 200410300002, Handbook of Recommended Practices for Questionnaire Development and Testing in the European Statistical System.
2. Questionnaire design; CDC
3. https://www.iarc.fr/en/publications/pdfs-online/epi/cancerepi/CancerEpi-18.pdf
4. http://cahnrs.wsu.edu/fs/wp-content/uploads/sites/4/2015/09/A-Step-By-Step-Guide-to-Developing-Effective-Questionnaires.pdf

Exercise

Based on observations given below frame the epidemiologically relevant question and try to develop a questionnaire to answer the question

Epidemiological Observation: A sudden rise in the deaths of cattle was observed in cluster of villages in Kolar district of Karnataka in the month of November 2014. The oozing of un-clotted blood was observed in dead cattle.

Diagnostic tests – An Epidemiological View

Dr. M. Nagalingam

ICAR – National Institute of Veterinary Epidemiology and Disease Inforamtics
Yelahanka, Bengaluru, Karnataka 560064

Introduction

In epidemiology, diagnosis plays a vital role in establishing decisions such as whether to treat (or implement a program) or to do nothing or to wait in addition to the importance of the disease. The tools for the veterinarian in arriving diagnosis include experience, factual knowledge, intuition as well as diagnostic tests. The outcome of the diagnostic process is a statement as to whether an animal is considered normal or not normal. This could relate to disease or infection status as well as to productive performance or quality of life from an animal welfare perspective. In this chapter we will see how a diagnostic test is evaluated and how it can be used in understanding epidemiology.

Diagnostic tests

A diagnostic test is any device or process designed to detect, or quantify a sign, substance, tissue change, or body response in an animal or human being. There is one more category called screening test which are used on clinically healthy animals. The diagnostic test is a more or less objective method for reducing diagnostic uncertainty. The outcome of the diagnostic process often is interpreted as a dichotomous variable as well, such as the animal having or not having the disease. But if the diagnostic device measures on a continuous scale, such as serum antibody levels or somatic cell counts, a cut-off value has to be determined so that the result can be condensed into a dichotomous scale. The problem with any cut-off point is that it is likely to result in overlap between healthy and diseased individuals with regard to test results. It is desirable to quantify the relationship between diagnostic test result and "true" disease status so that the clinician can take account of this uncertainty when interpreting test results. The usefulness of diagnostic tests, that is their ability to detect an animal with disease or exclude an animal without disease, is usually described by terms such as sensitivity, specificity, positive predictive value and negative predictive value (NPV).

To work out how well a diagnostic test performs, we need to compare it with a 'gold standard.' A gold standard is a test or procedure that is absolutely accurate. It diagnoses all diseased animals that are tested and misdiagnoses none. Once samples are tested using the gold standard and the test to be evaluated, a 2 × 2 table can be constructed, allowing test performance to be quantified.

Table 1 Contingency table showing disease and test details for evaluation of the test

	Disease		Total
Test	Positive	Negative	
Positive	(a)	(b)	(a+b)
Negative	(c)	(d)	(c+d)
Total	(a+c)	(b+d)	(a+b+c+d)

Sensitivity

The sensitivity of a test is the probability of the test to generate positive results among animals that actually possess the disease.

Sensitivity = No. of true positives/ (No. of true positives + No. of false negatives)

= a/(a + c)

A test with a high sensitivity is useful for 'ruling out' a disease if an animal tests negative. The mnemonic SnNout (highSensitivity, Negative test=rule out) is a useful way of remembering this principle.

Specificity

The specificity of the test is the probability of a test to generate negative results among animals that are genuinely free of the disease.

Specificity = No. of true negatives/ (No. of true negatives + No. of false positives)

= d/ (b + d)

A test with a high specificity is useful for 'ruling in' a disease if an animal tests positive. The mnemonic for remembering this is SpPin (high Specificity, Positive test, rule in)

Positive predictive value

It refers to the proportion of animals actually with the disease among all of the animals with positive test results. It answers the question: "If the test result is positive what is the probability that the animal actually has the disease?"

Positive predictive value = No. of true positives / (No. of true positives + No. of false positives)

= a/ (a + b)

If the prevalence of the disease is high, the predictive value of a positive test will also be high, but a good test should have a high predictive value even though the prevalence of the disease is low. Also a large difference in sensitivity makes a small difference in the predictive value of a positive test and that a small difference in specificity makes a big difference in the predictive value of a positive test. This means that the characteristic of a screening test described by specificity is more important in determining the predictive value of a positive test than is sensitivity.

Negative predictive value

It refers to the proportion of animals free of the disease among all of the animals with negative test results. It answers the question: "If the test result is negative what is the probability that the animal does not have disease?"

Negative predictive value = No. of true negatives/ (No. of false negatives + No. of true negatives)

= d/ (c + d)

Likelihood ratios

The dependence of predictive values on prevalence is a major disadvantage when a summary measure of a test's performance, when the test is applied in a population, is required. The likelihood ratio provides a suitable summary measure, which is independent of prevalence. It compares the proportion of animals with and without disease, in relation to their test results. **The likelihood ratio of a positive test result (LR+)** is the ratio of the proportion of affected individuals that test positive, and the proportion of healthy individuals that test positive.

LR+ = [a/ (a + c)]/ [b/ (b + d)]

The LR+ is therefore a quantitative indication of the strength of a positive result. The perfect diagnostic test would have an LR+ equal to infinity (detecting all true positives, and generating no false positives), and the best test for ruling in a disease is therefore the one with the highest LR+

The likelihood ratio of a negative test result (LR-) is the ratio of the proportion of affected individuals that test negative, and healthy individuals that test negative; that is:

LR- = [c/ (a + c)]/ [d/ (b + d)]

The perfect diagnostic test would have an LR- equal to zero (producing no false negatives, but detecting all true negatives), and the best test for ruling out a disease is therefore the one with the lowest LR-.

Likelihood ratios if used in combination with the initial expectation of the probability that an animal has a certain condition (= pre-test probability), a revised estimate of the overall probability of the condition (= post-test probability) can be calculated.

General rules about diagnostic tests

- Sensitivity and specificity are generally independent of prevalence.
- If the prevalence increases, positive predictive value increases and negative predictive value decreases.
- If the prevalence decreases, positive predictive value decreases and negative predictive value increases.
- The more sensitive a test, the better its negative predictive value.
- The more specific a test, the better its positive predictive value.

Prevalence estimation with diagnostic Tests

Tests produce false negatives and false positives, therefore any diagnostic test can only produce an estimate of the apparent prevalence. The apparent prevalence is the proportion of all animals that give a positive test result. It can be more than, less than or equal to the true prevalence. Estimates of the true prevalence can be obtained taking account of test sensitivity and specificity using the formula

True prevalence = [Apparent prevalence + (Specificity-1])/[Specificity+ (Sensitivity-1)]

Diagnostic strategies

Clinicians commonly perform multiple tests to increase their confidence. Multiple test results can be interpreted in parallel or series.

1. **Parallel** – the tests are performed at the same time and interpreted together.

Table 2. Parallel testing

Test A	Test B	Diagnosis
(-)	(-)	Negative
(+)	(-)	Positive
(-)	(+)	Positive

When two tests are used simultaneously, disease positives are defined as those who test positive by either one test or by both tests and disease negatives are defined as those who test negative by both tests When two (or more) tests are conducted in parallel, the goal is to maximize the probability that subjects with the disease (true positives) are identified (increase sensitivity). Consequently, more false positives are also identified (decrease specificity).

2. Serial – the tests are performed sequentially. The results of the first test usually determine whether the second test is still necessary or not. Only the positive cases are retested.

Table 3. Serial testing

Test A	Test B	Diagnosis
(+)	(-)	Negative
(-)	(+)	Negative
(+)	(+)	Positive

In parallel testing, there is a net gain in sensitivity but a net loss in specificity, when compared to either of the tests used but in serial testing, there is a net loss in sensitivity but a net gain in specificity, compared to either of the tests used

Screening and confirmatory testing

With a strategy of screening and confirmatory testing, as it is often used in a disease control scheme, the screening test is applied to every animal in the population to search for test-positives. This test should be easy to apply at a low cost. It has to be a highly sensitive test so that it misses only a small number of diseased or infected animals. Its specificity should also be reasonable, so that the number of false positives subjected to the confirmatory test remains economically justifiable. Negative reactions to the screening test are considered definitive negatives, and are not submitted to any further tests. Any positive screening test result is subjected to a confirmatory test. This test can require more technical expertise and more sophisticated equipment, and may be more expensive, because it is only applied to a reduced number of samples. But it has to be highly specific, and any positive reaction to the confirmatory test is considered a definitive positive.

Accuracy and precision

The accuracy of a test relates to its ability to give a true measure of the substance being measured. To be accurate, a test need not always be close to the true value, but if repeat tests are run, the average of the results should be close to the true value. The precision of a test relates to how consistent the results of the test are. If a test always gives the same value for a sample (regardless of whether or not it is the correct value), it is said to be precise.

Reliability

The value of a diagnostic test is also judged by its reliability, that is, the extent to which its results are stable. This can be explored by running the test two or more times on the same samples in the same laboratory under the same conditions, and assessing the repeatability of the results. Tests that are used in several laboratories (e.g., those that are recognized as international standards) also require their reproducibility to be determined.

Also, the characteristics of each disease should be known before either a testing strategy is developed or the results can be meaningfully interpreted.

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Epidemiologic Disease Investigation

Dr. Divakar Hemadri
ICAR – National Institute of Veterinary Epidemiology and Disease Inforamtics
Yelahanka, Bengaluru, Karnataka 560064

Disease investigation is an integral part of epidemiology. Epidemiological disease investigations are one of the most exciting and challenging tasks faced by an epidemiologist. These are required to explore the cause and source of the outbreak, which is frequently unknown. If the disease involved is a public health problem where in large number of people and animals are affected, timely intervention to control and contain the disease are quickly needed to not only to regain confidence of the general public but also to minimize any economic losses arising out of the disease epidemic. Disease investigation is a team effort and essentially each member of the team has specific role to play. It generally involves 8-10 steps, which are as follows:

- Confirming the existence of an epidemic or an outbreak.
- Verifying the diagnosis.
- Case finding and Data Collection
- Perform descriptive epidemiology
- Developing hypothesis
- Evaluating Hypothesis
- Implementing control/prevention measure
- Communicating findings

Unfortunately, disease investigation is not that simple and the paper describes difficulties associated such type of investigation with reference to India.

Applications of GIS and Remote sensing in Veterinary Epidemiology

Dr. Mohd. Mudassar Chanda

Scientist,

ICAR – National Institute of Veterinary Epidemiology and Disease Inforamtics
Yelahanka, Bengaluru, Karnataka 560064

I) GIS in Veterinary Epidemiology

Introduction

A geographic information system (GIS) is a system designed to capture, store, manipulate, analyse, manage, and present all types of spatial or geographical data in a computer. GIS is a system of computer software, hardware and data, and the personnel to enter, manipulate, analyse the data. The hardware component of computer is on which a GIS operates. The software components of GIS rely on the underlying Database Management System (DBMS). The data is the most important component of GIS comprising of geographic features and attribute data. Finally, the people who operate the GIS are very important in utilising the capabilities of a GIS system. Earlier, there were specialist to operate a GIS system and now it is used in every field starting from Geography to disease mapping field. Nevertheless, the full potential of GIS is not realised in the field of Veterinary Epidemiology in India. Remote sensing technology relies heavily on the GIS software and the details of which are discussed later.

Types of data in GIS

There are two types of data stored in the database of GIS. The attribute data gives information about the data like for example the livestock population. The spatial feature gives the information about where the feature is located in spatial domain (e.g. Livestock population in districts of South India). Spatial data can be either stored in raster or a vector format. A raster data is a continuous surface and the attribute data has rows and columns of number with a Digital Value/Number (DN) for each cell (Figure1). Units are usually represented as square grid cells that are uniform in size. The satellite images, aerial photography or scanned images can all be stored in raster format. The vector data are discrete features of spatial data and they can be of three different forms- points, lines and polygons (Figure 2, 3 & 4). Vector data are stored as x and y coordinates or a series of x & y coordinates.

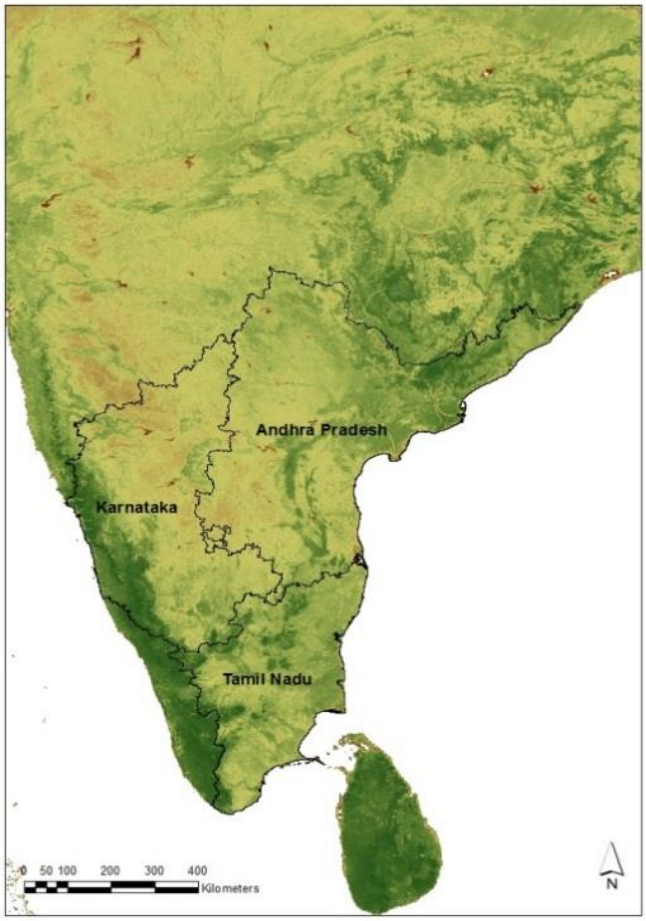

Figure 1: Satellite image in the raster format showing vegetation in South India.

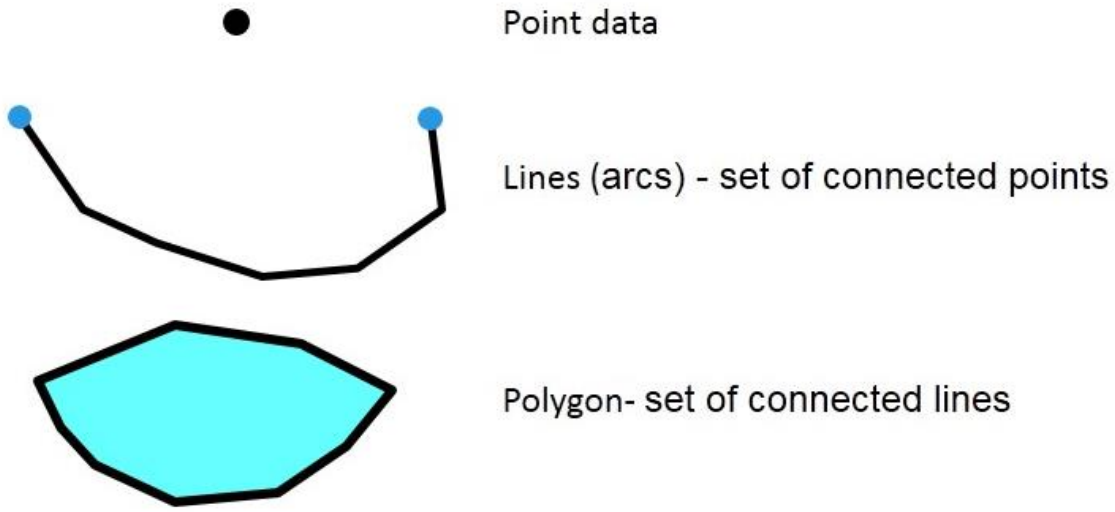

Figure 2: Types of vector data – point data, lines and polygon

Figure 3: Line map showing the major rivers flowing through South India

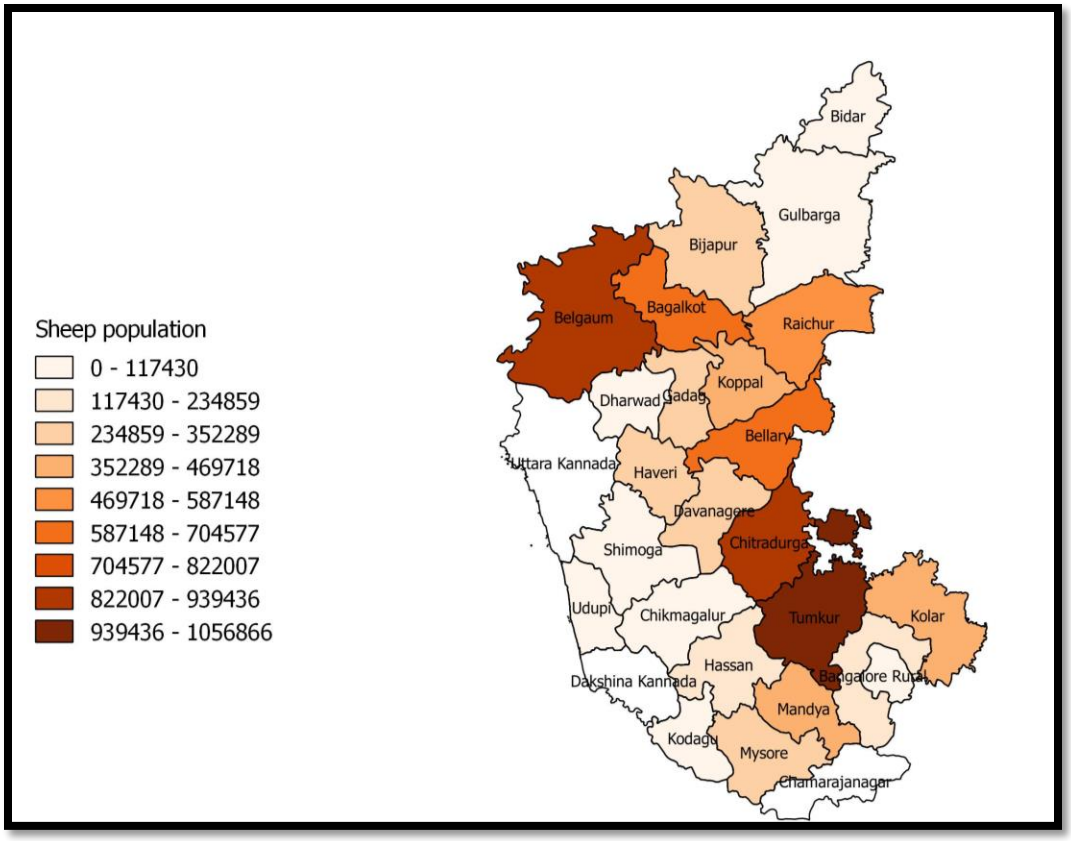

Figure 4: Polygon map showing Sheep population in Karnataka.

Application of GIS in Veterinary Epidemiology

There is so much data available which can be used in GIS platforms which can be padded up with epidemiological data for overall understanding of the spread of diseases. The data visualization using GIS is more helpful than displaying tables. The GIS can help us to understand the spatial features of data revealing hidden patterns, trends etc., which may not be apparent in spreadsheets. The application of GIS is not only restricted to better visualization but can also be used for many other purposes as discussed below. The GIS can be applied in the field of Veterinary Epidemiology for understanding and explaining the disease dynamics. The major application of GIS in Veterinary Epidemiology can be broadly grouped in these categories.

1. Field surveys to collect the epidemiological data using GPS
2. Mapping the point data and interpolation
3. Choropleth mapping
4. Overlaying disease data with other layers
5. Disease modelling
6. GIS in decision support system for economically important livestock diseases

1. **Field surveys to collect the epidemiological data using GPS**: Global Positioning System (GPS) based collection of epidemiological enables to map the location of the outbreaks (Figure 5). The point data can be of help for further analysis in GIS by calculating distances from the sample collected. The distance to water bodies or forest or any other location enables to understand the disease epidemiology.

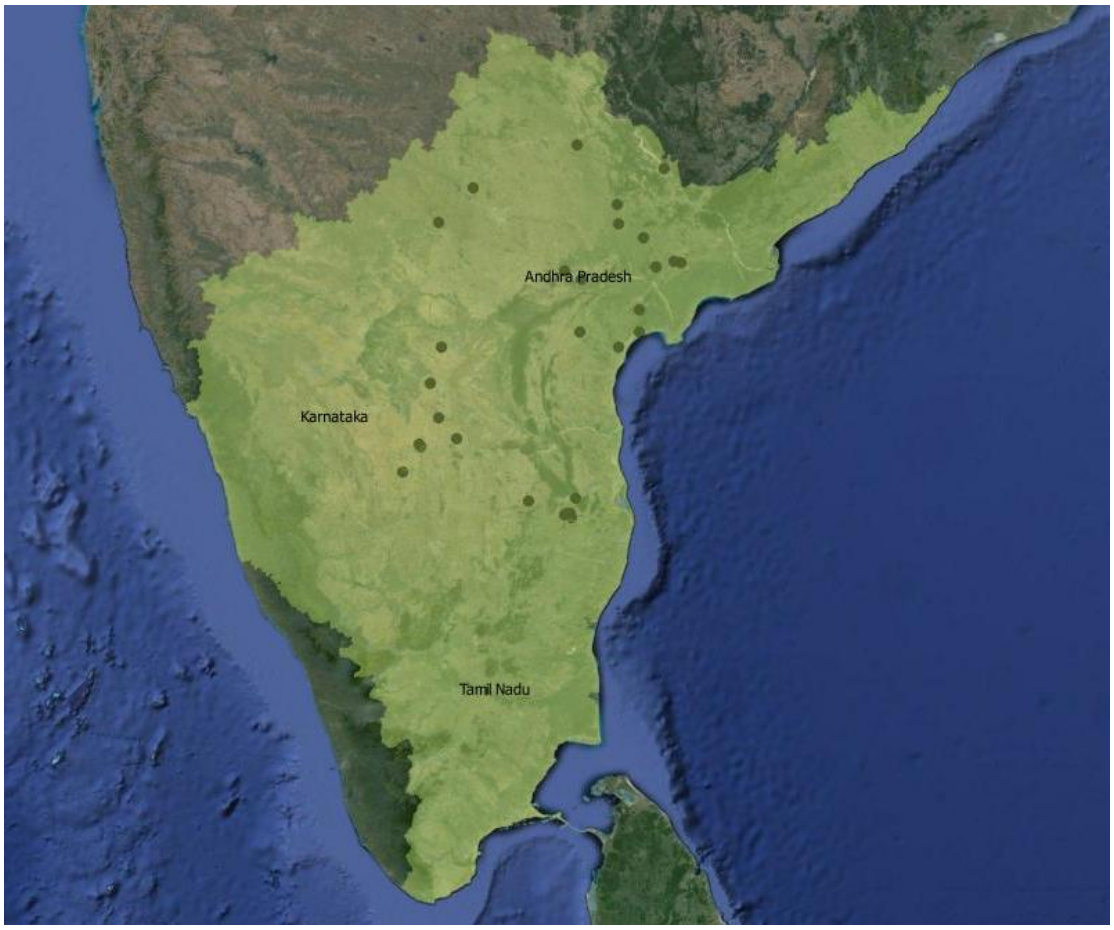

Figure 5: Point data map showing locations of a disease outbreak in South India

2. **Mapping the point data and interpolation**: The other aim of collecting point data is for interpolation in the areas where the samples have not been collected for identification of risk areas. There are many interpolation techniques and it depends on the type of data collected and the disease under study. The interpolated risk maps can be of great help in planning future surveillance activities and also in strategizing disease control measures.
3. **Choropleth mapping**: A map that uses graded differences in shading or colour or the placing of symbols inside defined areas on the map in order to indicate the average values of some property or quantity in those areas. A choropleth map showing exotic & crossbred sheep population in three states of South India (Figure 6).

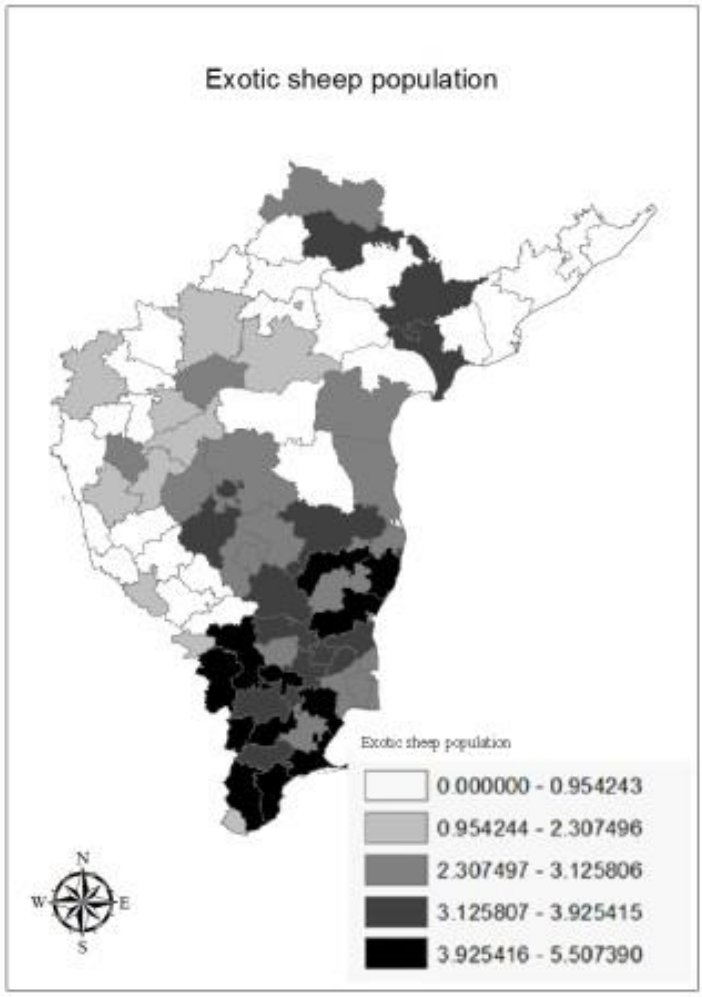

Figure 6: The Choropleth map showing the population of Exotic and crossbred sheep in three South Indian states. (Darker the color more the density)

4. **Overlaying disease data with other layers**: GIS overlaying can be used to understand the epidemiology of disease and the probable factors responsible for the outbreaks. The point map can be overlaid with other layers such as soil map, river map, vegetation map etc. Outbreak data (point map) is overlaid with river map (line data) and the districts (polygon) of three Southern states (Figure 7).

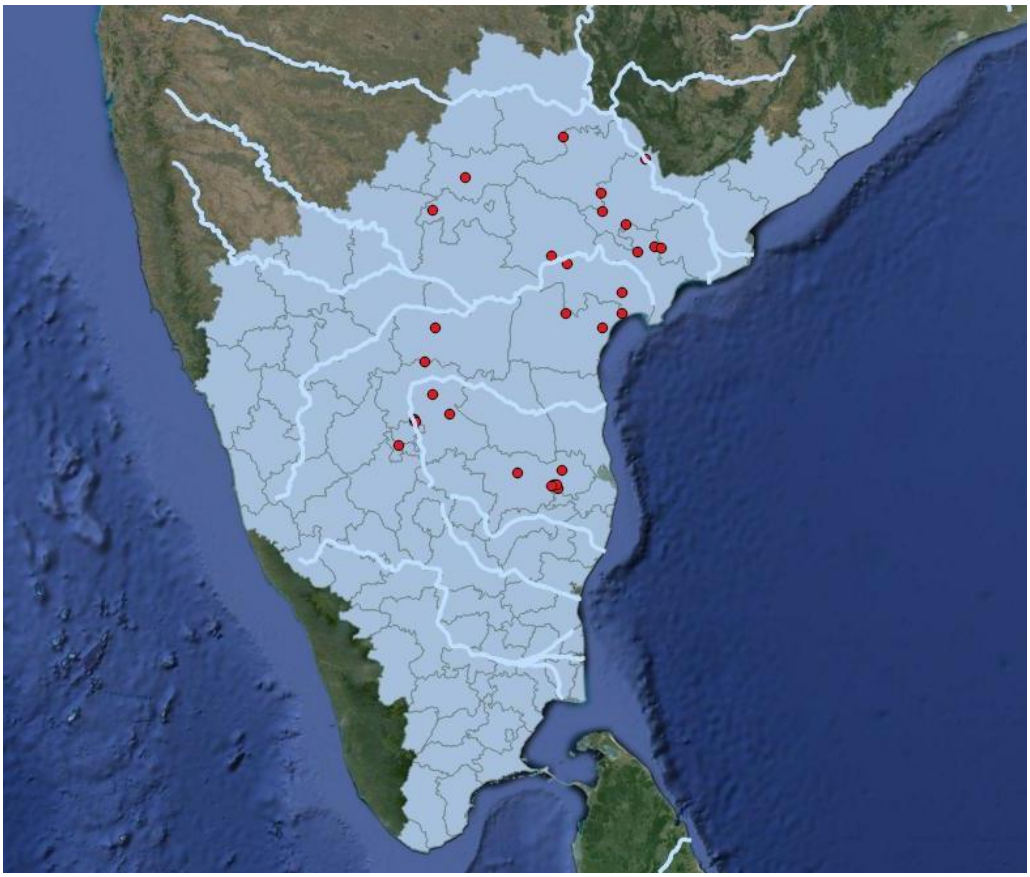

Figure 7: Over layering of different spatial data. The dots (Point map) indicate location of the outbreaks, line (line map) indicates the rivers flowing near the outbreaks regions and the districts (polygon map) shows the location of districts with respect to the outbreaks location.

5. **Disease modeling:** GIS can also be used to quantify the relationship between any disease data and the environmental factors (land cover, precipitation etc.) for better understanding of the disease epidemiology. Spatial and ecological data have been combined to understand the risk factors for vector borne diseases of human importance, but not much applied in animal diseases.
6. **GIS in decision support system for economically important livestock diseases:** The GIS can also be used in the decision support system (DSS) to inform the disease managers about the risk areas and suitable control measures can be advised. The GIS based decision support system in real time using websites which will be very helpful for the policy makers. The GIS based decision support is very common in the field of road transportation, water management, meteorological forecasting etc., but not commonly employed in the field of animal diseases. The GIS based decision support system can be very useful for development of disease early warning system which can be helpful for effective planning and control of economically important livestock diseases.

II) Remote sensing and its application in Veterinary Epidemiology

Remote sensing has been defined as "the science and art of obtaining information about an object, area, or phenomenon through the analysis of data acquired by a device that is not in

contact with the object, area, or phenomenon under investigation"(Lillesand, Kiefer et al. 2004). The basic idea behind remote sensing is the fact that every object, area or phenomenon reflects and emits energy at specific and distinctive wavelengths of the electromagnetic spectrum. Use of remote sensed data for environmental purposes is restricted to visible, infrared, and microwave regions of this spectrum. The images acquired through satellites are processed to identify or monitor features for further use in environmental studies or epidemiological studies.

Remote sensed imagery obtained from orbiting satellites used to display and compare vegetation, ground temperature and moisture. The images are captured for specific location at a known time and date and therefore the changes (eg. Vegetation or temperature) can be compared over time. The remote sensed images can be integrated in to GIS for further analysis and interpretation. The resolution of the satellite images depends on the sensors and mirrors along with satellite altitude. For example the LANDSAT-TM has a resolution of 30m for bands 1-5 & 7 and 120m for the thermal band 6. The resolution of the SPOT is 10m or 20 m depending on the system being used. The minimum unit of the captured satellite image is known as pixel. The pixels are arranged in regular rows (x) and columns (y) with a digital number (z) of the intensity of the electromagnetic energy measured for the area of the ground. The captured remote sensed image can then be processed in GIS software to highlight the contrast between different structures or vegetation. The application of remote sensed images is common in studying epidemiology of human diseases like leishmaniasis, malaria, Dengue. For example the development of major irrigation and hydroelectric schemes on the Mahaweli River near Kandy in Sri Lanka has resulted in drastic increase in malaria incidence and such sites can be monitored using satellites (LANDSAT or SPOT).

Remote sensed variables as surrogates of meteorological variables

In past, meteorological variables have been used as predictor variables in modelling of human and animal diseases and the quantified relationship is used for making predictions in unknown areas. Although, meteorological variables give specific parameters like air temperature or relative humidity which are important for determining the abundance of vectors for disease, but there is lack of information for all the places due to practical difficulties in establishing meteorological stations. The Remotely sensed variables have the advantage of fine spatial (up to 200 meters) and temporal resolution and these variables have been used as surrogates for meteorological variables in establishing relationship between diseases or disease vectors and, thus can be effectively be used in risk mapping and forecasting of important diseases of livestock and human health. One such example is use of MODIS (MODerate-resolution Imaging Spectroradiometer), which is freely available for research community and available at 1km resolution (each image can capture area of 1km). The temperature and reflectance layers acquired through processing of MODIS data can be further processing in GIS software's to calculate the Normalized Difference Vegetation Index (NDVI), Enhanced Vegetation Index (EVI), day time land surface temperature (dLST) and night time land surface temperature (nLST). NDVI is correlated with soil moisture, rainfall and vegetation biomass, coverage and productivity (Campbell 2002). The NDVI is very useful for monitoring the greening and senescence of habitats. The NDVI is useful in predicting and modeling of human and livestock disease and vector abundance. It has been used in the past for tsetse flies and human trypanosomiasis, Rift Valley fever and also used in mapping Theileria Parva in Africa.

Remote sensed variables as predictor variables in modeling and risk mapping of livestock diseases

A risk map is a data visualization tool for communicating specific risks an organization faces. Disease risk maps are produced by quantifying relationship between environmental variables (ground station meteorological data or remote sensed data) and can be used by policy makers to develop disease controlling strategies.

Remote sensed variables have been used in past as predictor variables in modeling and forecasting of diseases of public health importance (Rogers, Randolph et al. 2002). The use of remotely sensed variables in developing risk maps and modeling livestock diseases is constantly used in developed countries. The remote sensed variables can also be used in forecasting livestock diseases by improving the disease surveillance by effective use of sensitive diagnostic and entering the data at village level. The improved surveillance and data gathering process at village level will enable to extract the co-ordinates for villages, which can be used in extracting the remote sensed data for modeling and forecasting at fine scale.

Land cover maps using remote sensing and its use in identification of risk factors for animal diseases

Land cover refers to what covers the surface of the earth for example; crops, forests, water bodies, urban areas etc. The land cover maps are useful in identification of risk factors for both directly transmitted diseases and also indirectly transmitted diseases. Certain land cover types (for example water bodies) may be important for diseases such as Fascioliasis (parasitic disease affecting ruminants) and certain land cover types (forests) may be important in determining the presence of tick borne animal diseases. The land cover map derived from satellite data and validated ion ground can be effectively used in identifying the risk factors for many livestock diseases and control measures can be targeted in high risk areas. The remote sensed data can be integrated in the GIS software and all the application discussed in the previous section can be extended to utilise its potential.

Conclusions

Geographical Information system are powerful system for collection, storage, retrieval and analysis of spatial data. The application of GIS in the field of Veterinary Epidemiology has not been harnessed to its full potential in India. The applications are not limited to the areas discussed above, but it can be applied to other areas depending on the disease under study. There are many software's (both open source and licensed) available in the market which can be used for GIS application in Veterinary Epidemiology. The application of remote sensing in Veterinary Epidemiology has not been fully utilised in India and future studies should harness its full potential which will help improved surveillance and control of livestock diseases.

Further reading

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Introduction to livestock disease Economics

Dr. G. Govindaraj
ICAR – National Institute of Veterinary Epidemiology and Disease Inforamtics
Yelahanka, Bengaluru, Karnataka 560064

Introduction

The effect of disease in animals reduces the efficiency with which inputs are converted into outputs. Besides this there are several direct and indirect effects, of which, some can be valued or quantified easily and some are difficult to quantify. Besides quantification using the reliable data collected from primary or secondary sources, the effect of a disease can be modeled by implying certain assumptions like increased death rate, lower yield, decreased body weight, increased calving/kidding interval etc. It is also sometimes referred as simulation assessment of the impact of the disease.

Disease impact

Any disease in animals has direct and indirect impacts on the performance of animals and on animal keepers. The direct impact can be further classified into visible and invisible impacts. The visible impacts can be easily quantified. Eg. mortality loss, reduction in milk, wool reduction in draught power, money expended on vaccines and veterinarians fee. The invisible impacts due to a disease are reduced fertility and changed herd structure. The quantification of invisible losses can be made using certain models and assumptions since the exact information/data is not usually available and are difficult to obtain.

The indirect impact includes societal and financial impact. The non-disposal of dead animals in the scientific manner has impact on environment through release of harmful gases or spread of disease to disease free herds/animals in the neighborhood. The death of animals in large numbers in general or death of certain breeds/ species due to outbreaks in a certain geographical region has variable societal impact. The indirect impact like change in methane gas emission levels, change in dung availability and its impact on agriculture and allied activities productivity, change in carrying capacity etc., also affects the society at large due to disease in animals.

The death of animal also affects the availability of animal products like milk to the farm family especially in subsistence agriculture families. It also affects the human nutrition and thereby reduces the longevity. It also ends up in various social problems in the long run. The indirect impact of zoonotic diseases affects humans and has larger societal ramifications.

The indirect financial impact includes lesser price for the diseased animals in the market or lower value for the normal animals also in a particular locality due to an outbreak. The price of the complement and substitute goods also changes due a disease outbreak and hence the price effect should be a part of impact analysis.

All the above impacts has to be quantified in a scientific manner so as to arrive the overall impact of the disease. Quantifying the direct impact of any disease is relatively easy compared to quantifying the indirect impact. However, with use of implicit assumptions and indirect valuation methods the overall impact can be quantified.

Information required for assessing the impact of a disease

The foremost information required is about the disease itself, i.e.

a) Whether the disease is bacterial or viral or parasitic or combination thereof or any other agent?
b) Incidence rate of the disease
c) Duration of infection

ii) Diseased animal information

a) The species information (Eg. bovine, small ruminants, poultry, pig, horse, etc.)
b) The breed details (Eg. indigenous, crossbred, exotic)
c) The age and sex of the diseased animal
d) The susceptibility information agewise, sexwise, breedwise

iii) The physiological parameters at normal and diseased state

iv) Productive and economic traits at normal and diseased state

Indicators required for assessing the losses

The indicators required for assessing the losses due to animal diseases may vary for macro (national) or micro (farm) level estimation. The important indicators required for macro level disease impact estimates are as follows:

a) The number of animals died in different species and across different ages
b) The number of animals culled due to disease in different species and across different ages
c) The number of animals disabled due to disease in different species and across different ages
d) The productivity loss in animals like milk loss/day/animal
e) The number of days of milking loss in a lactation etc.
f) The number of days of draught power loss due to a disease
g) Information on decreased levels of fertility

Besides, the basic requirements listed above the economic information are also necessary to assess the impact of the disease. For time series disease impact assessment, the data on different time periods are necessary whereas for the cross section analysis the information during a particular time on different variables are required.

Some of the very important economic information required are given below:

a) Price of milk, wool, meat, etc.
b) Price of live animal for different breeds, for different age groups and for different sex
c) Price of the culled animal for different breeds, for different age groups and for different sex
d) Price of disabled animal across breeds, age and sex
e) Rates of bullock labour per day
f) Rates of hired labour per day for male and female

Conclusion

The analysis of disease losses from different angles helps the farmers as well as policy makers and planners. The estimation of losses species-wise tells farmers which species are more susceptible to diseases and by how much and thus they can manage to reduce losses in livestock. Similarly, age or herd size-wise estimate of losses suggest them at what age the animal is more susceptible. The disease loss information also help policy makers and planners to decide which disease ranks first in terms of losses so that they can get due importance in policy planning.

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

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Compiled & Edited
By
Dr. Md. Mudassar Chanda
Dr.V.Balamurugan
Dr. G. Govindaraj
Dr. Jagadish Hiremath
Mrs. Anusha Alamuri