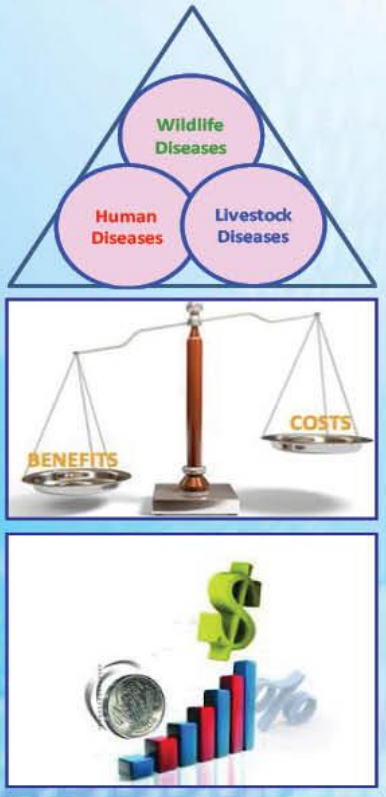
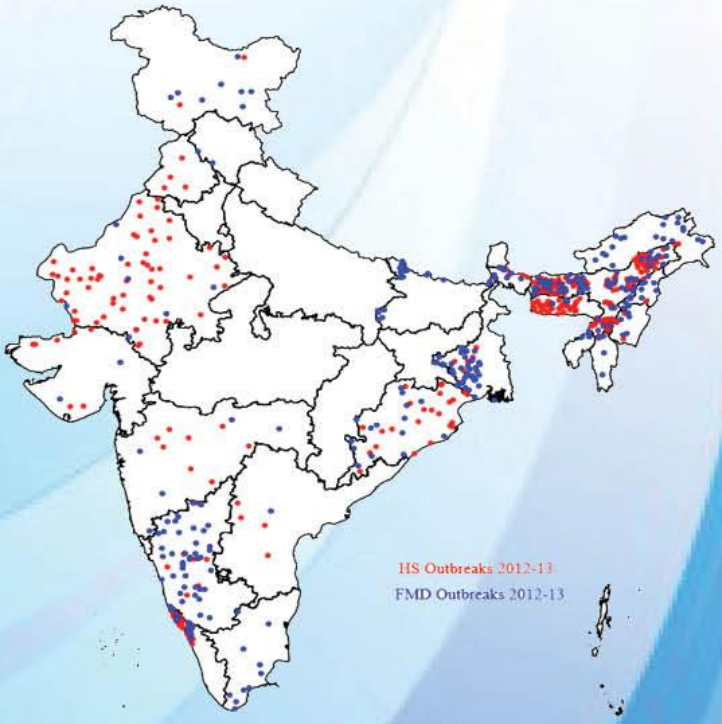
Model Training Course
On
Epidemiology and Impact Assessment of Livestock Diseases: Concepts, Application and Strategies

14- 21 December, 2015





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

(An ISO9001:2008 Certified Institute)

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Epidemiology and Impact Assessment of Livestock Diseases: Concepts, Application and Strategies

14 – 21 December, 2015

Training Manual

Compiled and Edited by

G. Govindaraj
P. Krishnamoorthy
R. Sridevi
V. Balamurugan

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Disclaimer

No responsibility is assumed by the Course Director and Co-Course Directors for the statement made by the authors in this training manual

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G. Govindaraj
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Model Training Course

on

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14 -21 December, 2015

at

ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), Yelahanka, Bengaluru-560064.

Programme Schedule

Date	Time	Presentation Topic	Resource persons
14.12.15	9.00 -9.30am	Registration	Dr.G.Govindaraj / Dr.R.Sridevi/ Dr.P.Krishnamoorthy/ Dr.V.Balamurugan
	9.30 -10.30am	Inauguration ceremony	
	10.30-11.00am	Pre -Training Evaluation	Dr.P.Krishnamoorthy / Dr.R.Sridevi
	11.00-11.15am	High Tea	
	11.15- 12.15pm	Introduction to Basic Epidemiology	Dr. H.Rahman / Dr.M.R.Gajendragad
	12.15-1.15pm	Biosafety practices in Veterinary Diagnostic Laboratories	Dr.G.S.Desai
	1.15-2.00pm	Lunch Break	
	2.00-3.15pm	Outbreak Investigation and generation of scientific reports	Dr. D.Hemadri
	3.15-3.30pm	Tea Break	
	3.30-5.00pm	Glossary of Epidemiology	Dr.P.Krishnamoorthy
15.12.15	9.30-10.30am	Types of Epidemiological studies for livestock disease investigations	Dr.D.Hemadri
	10.30-10.45am	Tea break	
	10.45-11.45am	Diagnostic Tests in Epidemiology	Dr.M.Nagalingam / Dr.V.Balamurugan
	11.45-1.15pm	Sampling techniques and sample size estimation for Epidemiological studies	Dr.K.P.Suresh
	1.15-2.00pm	Lunch break	
	2.00-3.00pm	Clinical sample collection and their management	Dr.Rajeswari Shome
	3.00-3.15pm	Tea break	
	3.15-4.15pm	Epidemiology and control programme of Peste des Petits Ruminants (PPR) in India	Dr.V.Balamurugan
	4.15-5.00pm	Descriptive and spatio - temporal Epidemiology	Dr.G.B.M.Reddy
16.12.15	9.30-10.30am	Epidemiology of Brucellosis and Control Programme in India	Dr.Rajeswari Shome
	10.30-10.45am	Tea Break	

	10.45-12.00	Clinical Epidemiology of livestock diseases	Dr.K. Rajkumar (Guest Faculty)
	12.00-1.15pm	Importance of Surveillance & monitoring : Role of Field veterinarians	Dr.M. Rajasekhar (Guest Faculty)
	1.15-2.00pm	Lunch break	
	2.00-3.00pm	Impact of livestock diseases- An introduction	Dr. G. Govindaraj
	3.00-3.15pm	Tea Break	
	3.15-5.00pm	Introduction to open source epidemiological software and its applications (Practical)	Dr. P. Krishnamoorthy
17.12.15	9.30-10.30am	Economic Impact of FMD in Indian Livestock	Dr. G. Govindaraj
	10.30-10.45am	Tea break	
	10.45-12.15pm	Methodologies for impact assessment of Livestock diseases and loss projection methods	Dr.Lalith Achoth, KAVFSU (Guest Faculty)
	12.15-1.15pm	Importance of Laboratory investigations in Epidemiological studies	Dr. R. Sridevi
	1.15-2.00 pm	Lunch break	
	2.00- 3.00pm	Loss assessment based on secondary data -PPR in Sheep in India	Dr.G.Govindaraj
	3.00-3.15pm	Tea break	
	3.15-4.30pm	Applications of GIS and Remote Sensing in Veterinary Epidemiology (Theory & Practical)	Dr. M. Chanda
	4.30- 5.00pm	Demonstration of forecasting/ forewarning software for livestock diseases (NADRES) (Practical)	Dr.D.Hemadri/Dr.S.S.Patil
18.12.15	9.30-10.30am	Epidemiology of Haemorrhagic Septicaemia & Anthrax	Dr.S.B.Shivachandra
	10.30-10.45am	Tea break	
	10.45-11.45am	Current Status of Veterinary Epidemiology in India	Dr.L.Gunaseelan (Guest Faculty)
	11.45-1.15pm	Epidemiology of Parasitic diseases in India	Dr.P.P.Sengupta/ Dr.Siju Susan Jacob
	1.15-2.00pm	Lunch break	
	2.00-3.45pm	Economics of mastitis, ketosis and sheep pox	Dr.B.Ganeshkumar (Guest Faculty)
	3.45-4.00 pm	Tea Break	
	4.00 -5.00pm	Vaccine Epidemiology	Dr.J.Hiremath
19.12.15	9.00 -5.00 pm	Field Visit for primary data collection to assess loss due to diseases	Dr.G.Govindaraj / Dr.P.Krishnamoorthy/ Dr.Yogesharadhaya

20.12.15	9.00-5.00pm	Field Visit for epidemiological investigation of livestock diseases	Dr.G.Govindaraj / Dr.P.Krishnamoorthy/ Dr.Yogesharadhaya
21.12.15	9.30-10.30am	Data feeding, checking, cleaning and assessment of losses (Practical)	Dr.G.Govindaraj
	10.30-10.45am	Tea Break	
	10.45-11.45am	Data feeding, checking, cleaning and assessment of losses (Practical)	Dr.G.Govindaraj
	11.45-1.15pm	Bacterial mapping of Mastitis	Dr.B.R.Shome
	1.00-2.00pm	Lunch Break	
	2.00-3.15pm	Post Training Evaluation	Dr.P.Krishnamoorthy / Dr.R.Sridevi
	3.15-3.30pm	Tea break	
	3.30-5.00pm	Valedictory Function	

1. Introduction to Basic Epidemiology

M. R. Gajendragad
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Epidemiology, in simple terms, is defined as the study of disease in a population and the factors that affect the occurrence of the disease. Literally, ***epi*** means upon; ***demo*** means people and **logo** means discoursing. It is basically an observational study and deals with place, time and population of a disease. Although not in the present form, the study of epidemiology is the oldest form of medical science used to understand the diseases in a population. The classical example of its use was controlling the epidemic of cholera in London in 1854. However, it can be traced back even to the observations of Jenner on Pox diseases.

Epizootiology is the term used for study of diseases of livestock, which not only covers all the aspects of Epidemiology but also the productivity.

The entire study of epidemiology rests on three aspects *viz.*, the host, the agent and the environment. The interactions of these three form the foundation of epidemiology called **"epidemiological triad"**.

Epidemiology is the scientific tool for administrators and policy makers to plan a control strategy.

Epidemiology observes and records a disease event, describes distributions, measures the associations and provides logical insight of the disease.
Broadly, the following will be discussed during the lecture

1. What is disease?
2. What are the categories of the diseases
3. What is epidemiology
4. What are the disease risks
5. Why do we need epidemiology
6. Need for epidemiology
7. Uses of epidemiology
8. Common types of epidemiological studies.

2. Biosafety Practices in Veterinary Diagnostic Laboratories

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Reducing the risk of emerging and re-emerging animal diseases is important in order to control their direct and indirect effects: from their obvious detrimental impacts on animal and human health to their broader economic implications in terms of the lost revenues and wider societal costs resulting from disease outbreaks. Approximately 75% of emerging animal diseases are zoonotic, meaning that they can be passed between humans and animals and vice versa, and many have the potential to be disabling or even fatal if left untreated. In the last decade, disease outbreaks have led to the culling of hundreds of millions of animals and have incurred huge costs. Avian influenza outbreak has been estimated to have led to the culling of 200 million birds in Asia alone, with losses of more than 10 billion US dollars for the region's poultry sector. The 2006 outbreak of Classical Swine Fever in Germany, led to the imposition of trade restrictions that amounted to a cost of 250-300 million US dollars. The cost of 2007 Australian outbreak of Equine Influenza to the industry an estimated 1 billion Australian dollars and government expenditures for quarantine measures and financial support totalling over 300 million dollars. The World Bank has estimated that the combined losses in trade, tourism and tax revenues due to animal disease outbreaks have amounted to approximately 200 billion dollars over the past decade. Hence, with current trends of human development, globalisation and climate change increasing the likelihood of emerging and re-emerging disease outbreaks, more robust surveillance, prevention and control measures will be needed in order to prevent future crises.

Surveillance and diagnosis of clinical cases of animal disease are necessary to determine the existence or introduction of a disease and laboratory testing is a crucial part of these surveillance programmes. Therefore, veterinary diagnostic laboratories are the backbone of disease control programmes administered by the veterinary services of a country. These diagnostic laboratories have biocontainment facilities, which allow them to work with highly contagious or controlled and/or zoonotic agents. In an effort to innovate newer methods of disease detection and to understand the disease dynamics in animals continued research is also carried out in these veterinary biocontainment laboratories. The reliability and expertise of the nationwide laboratory system is the strongest backbone of the animal health and public health system of the country.

Laboratories that handle pathogens have special requirements in terms of ensuring health and safety. The issues are protection of the personnel who work with the pathogens or at least in areas where the pathogens are handled or stored and protection of the environment and the public at large from contamination that could result from release of pathogens from the laboratory. The latter includes preventing the intentional release of pathogens to commit acts of bioterrorism. In addition to biological hazards due to the presence of infectious material, biological laboratories can pose chemical and physical hazards as well, and therefore the biosafety recommendations include measures to protect personnel from those hazards and to ensure their safe use, storage, management and disposal. Hence, 'biosafety' refers to the measures in place to protect humans and the environment while ' biosecurity' refers to the measures that protect the pathogens from damage and wrongful removal (accidental or intentional). In other words, laboratory biosafety describes the principles and practices for the

prevention of unintentional release of or 98 accidental exposure to biological agents and toxins.

Microbial agent/toxin specific laboratory risk assessments are used to identify the specific biosafety and biosecurity measures needed to contain and work safely in a laboratory or animal facility. The common practice of linking a biological agent to a specific level of biocontainment arises from the concept of identifying biological agents and toxins as biohazards and classifying the individual agents into one of four risk groups based on the potential to cause disease in an individual and in a community. Independent of the biological agent "risk group" classification process, biosafety level designations (alternately termed physical containment levels) were historically developed to characterise laboratories based on a composite of physical design features, facility construction, equipment, operational procedures, and laboratory practices required for working safely with the range of biological agents and toxins that pose varying levels of risk to individuals and to a community. The specific biosafety and biosecurity measures are identified during a biological risk assessment which takes into consideration a laboratory's organisation, the facility, and the surrounding environment in which the biological agent or toxin is to be handled.

Classification of Disease Causing Pathogens

Risk Group WHO	Risk Group OIE
1. A microorganism that is unlikely to cause human or animal disease. (no or low individual and community risk).	Enzootic disease organisms, no official control.
2. A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited. (moderate individual risk, low community risk).	Exotic or enzootic organisms, Low risk of spread, Official control, Not vectored, Species specific, Limited economic significance.
3. A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available (high individual risk, low community risk).	Exotic or enzootic organisms, Moderate risk of spread, Official control, May be vectored, Quarantine applied, Severe economic significance.
4. A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available (high individual and community risk).	Exotic or enzootic organisms, High risk of spread, Official control, May be vectored, Quarantine applied, Movement controls, Extremely severe economic significance.

The most important feature of biosafety is containment. There are three forms of containment:

- Primary Containment
- Secondary Containment
- Tertiary Containment

The primary containment is the first barrier between agent and man, the secondary containment is the barrier between agents and environment and a tertiary containment represents an additional organisational barrier. Primary barriers pertain to equipment such as gloves, gowns, masks, biosafety cabinets, respiratory protection, and positive-pressure ventilation suits as well as the use of good laboratory techniques. Secondary barriers are addressed through facility design with airtight rooms, air handling and filtration, air locks, showers, laundry, sewage treatment, waste disposal, sterilisers etc. Tertiary barriers deal with the physical operation with items such as walls, fences, and security, quarantine and animal exclusion zones. Due to the varying risk of biological agents, the facilities that handle these agents need to be designed and classified accordingly. The laboratories rooms should be equipped with biosafety cabinets of class II, supply corridors in animal facilities, storage rooms, washing rooms, safety corridors etc. Rooms, where aerosols are released on a regular basis, such as large animal rooms, necropsy rooms and dirty corridors should be surrounded by rooms with a lower contamination level.

Many infectious pathogens are transmitted by the aerosol route of exposure. In addition, aerosols of infectious pathogens may contaminate surfaces or objects. These contaminated surfaces and objects may transmit the pathogens by contact and accidental ingestion. The contaminated objects may also be carried out of the laboratory or holding room and create a potential for spread of the pathogenic material outside of the laboratory. Biohazardous aerosols of concern in the laboratory setting are generated by a number of uses involving infectious material. Manipulation of samples or cultures containing pathogens can generate aerosols by the release of force into the sample. Simple procedures such as sonification, mixing, pouring and pipetting can create aerosols. In animal facilities, aerosols of infectious pathogens may be generated by infected animals breathing, sneezing or coughing respiratory pathogens. For animals that shed particular pathogens through excretion, disturbance of bedding or other materials containing excretions can generate large amounts of infectious aerosols. In particular, loose-housed animals or animals that are the natural host of the pathogen may create significant aerosols. In addition, procedures with infectious animals, particularly necropsies may create aerosols that must be contained. Living and dead infected animals can be sources of aerosols. Containing the aerosol at the source significantly reduces the risks from bio-hazardous aerosols. Primary containment includes biological safety cabinets, isolators, containment caging, containment caps for centrifuges, transfer containers. Containment can be accomplished by physical separation, directional airflow, or both. The laboratory air supply and control for the containment laboratories is maintained by HVAC (Heating, Ventilation and Air Conditioning) System.

Table 2. **Relation of risk groups to biosafety levels, practices and equipment**

RISK GROUP	BIOSAFETY LEVEL	LABORATORY TYPE	LABORATORY PRACTICES	SAFETY EQUIPMENT
1	Basic – Biosafety Level 1	Basic teaching, research	GMT	None; open bench work
2	Basic – Biosafety Level 2	Primary health services; diagnostic services, research	GMT plus protective clothing, biohazard sign	Open bench plus BSC for potential aerosols
3	Containment – Biosafety Level 3	Special diagnostic services, research	As Level 2 plus special clothing, controlled access, directional airflow	BSC and/or other primary devices for all activities
4	Maximum containment – Biosafety Level 4	Dangerous pathogen units	As Level 3 plus airlock entry, shower exit, special waste disposal	Class III BSC, or positive pressure suits in conjunction with Class II BSCs, double-ended autoclave (through the wall), filtered air

BSC, biological safety cabinet; GMT, good microbiological techniques

Basic practices and procedures recommended for all laboratories

1. Limit access to work areas. Close doors during work with research materials.
2. Do not eat, drink, smoke, or apply of cosmetics in the work area. Do not store food in refrigerators that contain laboratory supplies.
3. Wear laboratory coats (preferably disposable) when in work area, but do not wear them away from the work area.
4. Wear eye/face protection if splashes or sprays are anticipated.
5. Wear disposable gloves when handling viable materials. These should be disposed of as biohazardous waste.
6. Do not pipet by mouth.
7. Decontaminate all work surfaces after each working day. Decontaminate all spills of viable material immediately.
8. Wash hands with soap or detergent after handling viable. Do not handle telephones, doorknobs, or other common utensils without washing hands.
9. Minimize aerosol generation.
10. Report spills, exposures, illnesses, and injuries immediately.
11. Be familiar with written instructions for laboratory procedures and proper responses to emergencies.
12. Keep biohazard waste in covered containers free from leaks. Use orange or red biohazard bags as required by institutional procedure. Dispose biomedical waste appropriately.
13. Minimize the use of sharps. Dispose sharps in seperate sharp container.
14. Use Biosafety Cabinets Class II (either A2 or B2 depending on the type of work) for all infectious work and for the works that generate potenstial aerosols.
15. The laboratory door should be closed when work is in progress, and appropriate access restriction, warning or biosafety signage clearly visible.
16. No infectious material shall be discarded down laboratory sinks or any other drain.
17. Workers shall be appropriately trained and verified as competent to perform the tasks assigned.
18. Emergency response plans should be developed to deal with the biohazard of any safety or security incident.

19. Proper inventory of all the biological agents/material stored should be kept. Biological agents/material should be stored under lock and key and the access should be highly restricted.
20. Proper lab records should be maintained by all staff and should be produced for auditing.
21. The staff of laboratory should constantly interact with Biosafety Officer for trouble shooting, updation in laboratory protocols and any other laboratory related issues.

Biosafety Regulatory Framework in India

The Ministry of Environment and Forests (MoEF) has notified the Rules for the Manufacture, Use, Import, Export and Storage of Hazardous Microorganisms/Genetically Engineered Organisms or Cells 1989 (known as 'Rules, 1989') under the Environment (Protection) Act, 1986. These rules and regulations cover the areas of research as well as large scale applications of Genetically Modified Organism (GMOs) and products made there from throughout India. The rules also cover the application of hazardous microorganisms which may not be genetically modified. Hazardous microorganisms include those which are pathogenic to animals as well as plants (http://dbtbiosafety.nic.in/).

3. Glossary of Epidemiology

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The following are the terms used in epidemiology which has been arranged alphabetically and the important definitions are given.

A

Agent- A factor that is essential for a disease, chronic conditions, or injury to occur. Examples of agents include microorganisms, chemical substances, forms of radiation and in the case of injury, physical force. Agents can cause a health problem by either being introduced or present in excess or at deficient levels.

Antibody- Any of a variety of proteins in the blood that are generated to produce immunity against microorganisms or their toxins.

Antigen- a substance (usually protein) that induces a specific immune response (eg. Circulating antibody production.

Association- The statistical relationship between two or more events, characteristics or other variables.

Attack rate- A form of incidence that measures frequency of disease, chronic conditions or injury in a particular population for a limited time, such as during an outbreak. In calculating attack rates, the numerator is the number of new cases of a health problem during an outbreak, and the denominator is the population at the beginning of the period.

Attack rate, secondary- A measure of the frequency of new cases of a disease, chronic condition or injury among the contacts of known case-patients.

B

Bar chart- A visual display in which each category of a variable is represented by a bar. Bar charts are used to show variations in size among categories.

Bias- A systematic deviation from the truth; any trend in the collection, analysis, interpretation, publication or review of data that can lead to conclusions that are systematically different from the truth.

Box plot- A visual display that summarizes data using a "box and whiskers" format to show the minimum and maximum values (ends of the whiskers), inter quartile range (length of the box), and median (line through the box).

C

Carrier- An animal harboring the infectious agent for a disease and can transmit it to others, but does not show signs of the disease. A carrier may be asymptomatic (never show signs of the disease) or may show signs of the disease only during the incubation period, convalescence, or post convalescence. The period of being a carrier may be short (a transient carrier) or long (a chronic carrier).

Case- An instance of a particular disease, chronic condition, or type of injury. A variety of criteria may be used to identify cases and the epidemiological definition of a case is not necessarily the same as the ordinary clinical definition.

Case-patient- An animal in a case-control study which has the disease or health condition under investigation.

Case definition- A set of standard criteria for determining whether an animal has a particular disease or health condition. A case definition specifies clinical criteria and details of time, place, and animal.
Case fatality rate- The proportion of animal with a particular condition (case-patients) who die from that condition. In calculating case-fatality rates, the numerator is the number of animal died from the condition and the denominator is the total number of animals with the condition.
Cause of disease- A factor (characteristic, behavior, event, etc.) that directly influences the occurrence of a disease. Reducing such a factor in a population should reduce occurrence of the disease.
Census- The enumeration of an entire population, usually including details on age, breed, sex, etc.
Chain of infection- A process that begins when an agent leaves its source through a portal of exit, is conveyed by some mode of transmission and then enters through an appropriate portal of entry to infect a susceptible host.
Class- A grouping of observations of values of a variable. Classes are created for convenience in analyzing frequency.
Class boundaries- The values determining the upper and lower limits of a class. Class interval. The span of values of a continuous variable that lies between the class boundaries.
Clinical criteria- The symptoms and features of a disease that would be detected by physician analysis.
Clinical disease- A disease that has been manifested by its symptoms and features.
Cluster- An aggregation of cases of a disease or other health condition that are closely grouped in time and place. The number of cases may or may not exceed the number expected, and frequently the expected number is not known. Cases of cancer and birth defects are often investigated as clusters.
Cohort- A well-defined group of people who have had a common experience or exposure and are then followed up, as in a cohort study or prospective study, to determine the incidence of new diseases or health conditions.
Confidence interval- A range of values for a variable (e.g., a rate).
Confidence level- The proportion of similarly constructed confidence intervals that include the parameter of interest.
Confidence limits- The end points (i.e., the minimum and maximum values) of a confidence interval.
Contact- Exposure to a source of an infection.
Contagious- Capable of being transmitted from one person to another by contact or close proximity.
Control- The group of people without the health problem under study in a case-control study; a person in that group. For controls, investigators choose people who are as similar as possible to the cases, but without the health problem under study. In a case-control study, the control group is compared with the case group to determine associations between exposures and outcomes and to test hypotheses.
Cumulative frequency- In a frequency distribution, the number or proportion of cases with a particular value or less.

D
Database- a structured collection of data, organized so that it can be accessed easily by a range of computer software

Death-to-case ratio- The number of deaths attributed to a particular disease, chronic condition, or type of injury during a specified period divided by the number of new cases of that disease or injury identified during the same period.
Denominator- The lower portion of a fraction. Epidemiologists use fractions to calculate rates, or ratios. The denominator is usually the population at risk.
Determinant- Any factor that brings about change in a health condition or in other defined characteristics.
Disease- any departure, subjective or objective, from a state of physiological or psychological health and well-being.
Distribution- The complete summary of the frequency and pattern of the values or categories of a measurement. In epidemiology, distribution is the frequency and pattern of health-related characteristics and events in a population.
Dot plot- A visual display of the specific data points of a variable.
Droplet nuclei- The residue of dried droplets that is easily inhaled and exhaled and may remain suspended in air for long periods and be blown over great distances.
Droplet spread- The direct transmission of an infectious agent by means of the aerosols produced in sneezing, coughing, or talking.

E, F, G
Endemic health condition- A disease, chronic condition, or type of injury that is constantly present in a given geographic area or population group; may also refer to the usual prevalence of a disease or condition.
Environmental factor- An extrinsic factor, such as geology, climate, insects, sanitation, or health services, that affects an agent and the opportunity for exposure.
Epidemic (Syn: outbreak) - The occurrence of more cases of a particular type of disease, chronic condition, or injury than expected in a given area, or among a specific group of animals, over a particular period of time.
Epidemic curve- A histogram that shows the course of an outbreak or epidemic by plotting the number of cases of a disease, chronic condition, or injury according to time of onset.
Epidemic period- The time span of an epidemic.
Epidemiologic triad- The traditional model of infectious disease causation, which has three components: an external agent, a susceptible host, and an environment that brings the host and agent together so that disease occurs.
Epidemiology- The study of the distribution and determinants of health conditions or events in populations and the application of this study to control health problems.
Epidemiology, veterinary- the investigation of disease, other health related events and production in animal populations and the making of inferences from the investigation in an attempt to improve the health and productivity of the populations
Epidemiology, analytic- The aspect of epidemiology concerned with why and how a health problem occurs. Analytic epidemiology uses comparison groups to provide baseline data so that associations between exposures and outcomes can be quantified and hypotheses about the cause of the problem can be tested. Examples include cohort studies and case-control studies.
Epidemiology, applied (or field) - The application or practice of epidemiology to control and prevent health problems.
Epidemiology, descriptive- The aspect of epidemiology concerned with gathering, organizing, and summarizing data on "animal" (Who is ill?), "time" (When did they become ill?), and "place" (Where could they have been exposed to the illness?). This information is then used to conduct analytic epidemiology.

Evaluation- Systematic and objective examination of activities to determine how relevant and effective they are.
Exposed group- A group whose members have had contact with a cause of or possess a characteristic that is a determinant of, a particular health problem.
Exposure- Coming into contact with a cause of, or possessing a characteristic that is a determinant of, a particular health problem.
Frequency- The amount or number of occurrences, of a disease, chronic condition, injury, or other attribute or event in a population.
Frequency polygon- A graph of a frequency distribution in which values of the variable are plotted on the horizontal axis and the number of observations is plotted on the vertical axis. Data points are plotted at the midpoints of the intervals and are connected with a straight line.
Graph- A visual display of quantitative data arranged on a system of coordinates.

H
Health- A state of complete physical, mental, and social well-being and not merely the absence of disease or other infirmity.
Health indicator- Any of a variety of measures (e.g., mortality rate) that indicate the state of health of animals in a defined population.
Health information system- A combination of health statistics from various sources. Data from these systems is used to learn about health status, health care, provision and use of services and the impact of services and programs on health.
High-risk group- A group of animals whose risk for a particular disease, health condition or type of injury is higher than that of the rest of their community or population.
Histogram- A visual representation of the frequency distribution of a continuous variable. The class intervals of the variable are grouped on a linear scale on the horizontal axis and the class frequencies are on the vertical axis. Rectangles are drawn so that their bases equal the class intervals, and their heights correspond to the class frequencies.
Host- A person or other living organism that is susceptible to an infectious agent under natural conditions.
Host factor- An intrinsic factor (e.g., age, breed, sex and species) that influences an individual's exposure, susceptibility, or response to an agent.
Hypothesis- A supposition arrived at from observation or reflection that leads to refutable predictions; any conjecture cast in a form that will allow it to be tested and refuted.
Hypothesis, alternative- The supposition that an exposure is associated with the health condition under study. The alternative is adopted if the null hypothesis proves implausible.
Hypothesis, null- The supposition that an exposure is not associated with the health condition under study. The null hypothesis is the basis for most parametric tests for statistical significance.

I, J, K, L
Immunity, active- Resistance developed in response to an antigen (infecting agent or vaccine) and usually characterized by the presence of antibody produced by the host.
Immunity, herd- The resistance of a group to an infectious agent. This group resistance exists because a high proportion of animals in the group are immune to the agent. Herd immunity is based on the number of animal who are susceptible and the probability that they will come into contact with an infected animal. By vaccinating large number of animals in a population, health officials used herd immunity to control and eradicate the disease.
Immunity, passive- Immunity conferred by an antibody produced in another host. This type of immunity can be acquired naturally by young one from its mother or artificially by administration of an antibody-containing preparation (antiserum or immunoglobulin).

Incidence- A rate that measures the frequency with which a health problem, such as a new injury or case of illness, occurs in a population. In calculating incidence, the numerator is the number of new cases occurring in the population during a given period of time and the denominator is the total population at risk during that time.
Incubation period- The period following exposure, when pathologic changes are not apparent, and ending with the onset of symptoms of an infectious disease.
Infectivity- The proportion of animals exposed to an agent and become infected.
Inter quartile range- The central portion of a distribution, calculated as the difference between the third quartile and the first quartile. This range includes the middle one-half of the observations in the set, leaving one-quarter of the observations on each side.
Latency period- The period following exposure, when pathologic changes are not apparent and ending with the onset of symptoms of a chronic disease.

M
Mean, arithmetic- The measure of central location commonly called the average. The arithmetic mean is calculated by adding all the values in a group of measurements and dividing by the number of values in the group.
Mean, geometric- The mean or average of a set of data measured on a logarithmic scale.
Measure of association- A quantified relationship between exposure and a particular health problem. Commonly used measures of association include relative risk, rate ratio and odds ratio.
Measurement scale- The complete range of possible values for a measurement. An example is the set of possible answers to a question in a survey.
Median- The middle value in a set of numbers (or the average of two middle numbers) above and below which lie an equal number of values.
Midrange- The halfway point, or midpoint, in a set of observations. For most types of data, the midrange is calculated by adding the smallest observation and the largest observation and dividing by two. The midrange is usually calculated as an intermediate step in determining other measures.
Mode- The most frequently occurring value in a set of observations.
Monitoring- the routine collection of information on disease, productivity and other characteristics possibly related to them in a population.
Morbidity- the amount of disease in a population (commonly defined in terms of incidence or prevalence).
Mortality rate- A measure of the frequency of occurrence of death in a defined population during a specified time interval.
Mortality rate, age-adjusted- A mortality rate that has been statistically modified to account for the effect of different age distributions in different populations in a study.
Mortality rate, age-specific- A mortality rate limited to a particular age group. In calculating age-specific mortality rates, the numerator is the number of deaths in the age group and the denominator is the number of people in that age group.
Mortality rate, cause-specific- The mortality rate from a specified cause. In calculating cause-specific mortality rates, the numerator is the number of deaths attributed to a specific cause during a specified time interval in a population and the denominator is the size of the population at the midpoint of the time interval.
Mortality rate, crude- A population's mortality rate from all causes of death.
Mortality rate, sex-specific- A mortality rate among either males or females.

N

Natural history of disease- The course of a disease from the time it begins until it is resolved.
Necessary cause- A factor that must be present for a disease or other health problem to occur.
Normal curve- A bell-shaped curve, which results when a normal distribution is graphed.
Normal distribution- The symmetrical clustering of values around a central location. A normal distribution 1) is a continuous, symmetrical distribution with both tails extending to infinity; 2) has an identical arithmetic mean, mode, and median; and 3) has a shape that is completely determined by the mean and standard deviation.
Notifiable disease- A disease that, by law, must be reported to public health authorities upon diagnosis.
Numerator- The upper portion of a fraction.

O
Observational study- An epidemiologic study in which there is no intervention and nature is allowed to take its course. Changes or differences in one characteristic are studied in relation to changes or differences in others.
Odds ratio- A measure of association used in comparative studies to quantify the relationship between an exposure and a health outcome; also known as the cross-product ratio.
Ordinal scale- A type of measurement scale. Ordinal scales consist of qualitative categories whose values have a distinct order. The categories are qualitative in that there is no natural distance to be measured between their possible values.
Outbreak (Syn: epidemic) - Because the public sometimes perceives "outbreak" as less sensational than "epidemic," it is sometimes the preferred word. Sometimes the two words are sometimes differentiated, with "outbreak" referring to a localized health problem and "epidemic," to one that takes in a more general area.
Outbreak, common source- An outbreak in which animals are exposed to a common harmful influence, such as an infectious agent or toxin. The exposure period may be brief, or animals may be exposed over a period of days, weeks, or longer, with the exposure being either intermittent or continuous.
Outbreak, point source- A common source outbreak in which the exposure period is relatively brief so that all cases occur within one incubation period.
Outbreak, propagated- An outbreak that does not have a common source, but instead spreads from animal to animal.
Outcome(s) - Any or all of the possible results that may stem from exposure to a causal factor or from preventive or therapeutic interventions; all identified changes in health status that result from the handling of a health problem.

P, Q, R
Pandemic- An epidemic occurring over a very wide area (several countries or continents) and usually affecting a large proportion of the population.
Pathogen- an organism that produces disease.
Pathogenicity- The proportion of animals infected by an agent and then develops clinical disease.
Percentile- A set of cut points used to divide a distribution or a set of ranked data into 100 parts of equal area with each interval between the points containing 1/100 of the observations. For example, the 5th percentile is a cut point with 5% of the observations below it and the remaining 95% above it.

Pie chart-A circular chart depicting observed data and divided into "slices" that are proportional to the frequency of the categories of the variable assigned to them.
Population- The total number of animals of a given area or country. In sampling, the population may refer to the units from which the sample is drawn, not necessarily the total population of animals. A population can also be a particular group at risk.
Portal of entry- A pathway into the host that gives an agent access to tissue that will allow it to multiply or act.
Portal of exit- A pathway by which an agent can leave its source.
Predictive value positive- A measure of the predictive value of a reported case or epidemic; the proportion of cases reported by a surveillance system or classified by a case definition that are true cases.
Prevalence- The number or proportion of cases or events or conditions in a given population.
Prevalence, period- The amount of a particular disease, chronic condition, or type of injury present in a population over a period of time.
Prevalence, point- The amount of a particular disease, chronic condition, or type of injury present in a population at a single point in time.
Prevalence rate- The proportion of animals in a population having a particular disease, chronic condition, injury, or attribute at a specified point in time or over a specified period of time.
Proportion- A ratio in which the numerator is included in the denominator; the ratio of a part to the whole, expressed as a "decimal fraction"' (e.g., 0.2), a fraction (1/5), or a percentage (20%).
Proportion, attributable- A measure of the impact of a causative factor on the public health; the proportion of a disease, chronic condition, or injury that can be attributed to exposure to a particular factor.
Proportionate mortality- The proportion of deaths in a population attributable to a particular cause over a period of time. Each cause of death is expressed as a percentage of all deaths, and the sum the proportionate mortality for all causes must equal 100%. These proportions are not mortality rates because the denominator is all deaths instead of the population in which the deaths occurred.
Public health surveillance- The systematic, ongoing collection, analysis, interpretation and dissemination of health data. The purpose of public health surveillance is to gain knowledge of the patterns of disease, injury and other health problems in a community so that we can work toward controlling and preventing them.
Range- In statistics, the difference between the largest and smallest values in a distribution; in common use, the span of values from smallest to largest.
Rate- An expression of the relative frequency with which an event occurs in a defined population.
Rate ratio- A comparison of two groups in terms of incidence rates or mortality rates.
Ratio- The relative size of two quantities. A ratio is expressed by dividing one quantity by the other.
Relative risk- A comparison of the risk of a health problem in two groups.
Reservoir- The habitat in which an infectious agent normally lives, grows and multiplies. Humans, animals and the environment can serve as reservoirs.
Reliability- the degree of stability exhibited when a measurement is repeated under identical conditions, reliability therefore may be demonstrated by repeating a measurement.
Reservoir- an animate or inanimate object on or in which an infectious agent usually lives and which therefore is often a source of infection by the agent.
Risk- The probability that an individual will be affected by, or die from, an illness or injury within a stated time or age span.

Risk factor- An aspect of animal behavior or an environmental exposure or a hereditary characteristic that is associated with an increase in the occurrence of a particular disease, chronic condition, or injury.
Risk ratio- A comparison of the risk of a particular health problem in two groups.

S
Sample- A selected subset of a population. A sample may be random or nonrandom and representative or non-representative.
Sample, random- A sample of individuals chosen in such a way that each one has the same (and known) probability of being selected.
Sample, representative- A sample whose characteristics correspond to those of the original or reference population.
Scatter diagram (or Scattergram) - A graphic display of the relationship between two variables. A dot is plotted on the graph for each set of paired values for two continuous variables, with one variable plotted on the horizontal axis and the other plotted on the vertical axis.
Screening- the identification of unrecognized disease or defect in an apparently health population.
Seasonality- Change in physiological status or in the occurrence of a disease, chronic condition, or type of injury that conforms to a regular seasonal pattern.
Sensitivity- The ability of a system to detect epidemics and other changes in the occurrence of health problems; the proportion of animal with a health problem which are correctly identified by a screening test or case definition.
Skewed- A distribution that is asymmetrical.
Specificity- The proportion of animals without a particular disease, chronic condition, or type of injury who are correctly identified by a screening test or case definition.
Sporadic illness- An illness that occurs infrequently and irregularly.
Spot map- A visual display of the geographic pattern of a health problem. On a map of the area, a marker is placed to indicate where each affected animals' lives or may have been exposed. Spot maps can reveal clusters or patterns that provide clues to the identity and origins of the problem.
Spreadsheet- a computer software package providing a representation of a large rectangular area upon which data tabulation may be displayed and a variety of calculations performed.
Standard deviation- A statistical summary of how dispersed the values of a variable are around its mean. Standard deviation is equal to the positive square root of the variance.
Standard error (of the mean) - The standard deviation of a theoretical distribution of sample means of a variable around the true population mean of that variable. Standard error is computed as the standard deviation of the variable divided by the square root of the sample size.
Statistical inference- Generalizations developed from sample data, usually with calculated degrees of uncertainty.
Study, analytic- A study in which groups are compared to identify and quantify associations, test hypotheses, and identify causes. Two common types are cohort studies and case-control studies.
Study, case-control- An analytic study that compares a group of animals with a certain disease, chronic condition, or type of injury (case-patients) with a group of animals without the health problem (controls) to detect differences in characteristics such as exposure to an agent.
Study, cohort (Syn: follow-up, longitudinal and prospective study) - An observational analytic study in which enrollment is based on status of exposure to a certain factor or

membership in a certain group. Populations are followed and disease, death or other health-related outcomes are determined and compared.
Study, experimental- A study in which investigators identify the type of exposure that each individual (clinical trial) or community (community trial) has had and then follows the individuals' or communities' health status to determine the effects of the exposure.
Sufficient cause- A causal factor or collection of factors whose presence is always followed by the occurrence of a particular health problem.
Surveillance- the ongoing systematic collection and collation of useful information about disease, infection, intoxication or welfare in a defined animal population, closely integrated with timely analysis and interpretation of this information and dissemination of relevant results to those requiring them, including those responsible for control measures.
Survival curve- A curve that starts at 100% of the study population and shows the percentage of the population still surviving at successive times for as long as information is available. A survival curve may also be used to depict freedom from a health problem, complication or some other endpoint.

T
Transmission (of infection) - Any mode or mechanism by which an infectious agent is spread to a susceptible host.
Transmission, biologic- Indirect transmission by a vector in which the infectious agent undergoes part of its life cycle inside the vector before it is transmitted to the host.
Transmission, direct- Immediate transfer of an agent from a reservoir to a host by direct contact or droplet spread.
Transmission, indirect- Transfer of an agent from a reservoir to a host either by being suspended in air particles (airborne), carried by an inanimate intermediary (vehicle borne) or carried by an animate intermediary (vector-borne).
Transmission, mechanical- Indirect transmission by a vector in which the infectious agent does not undergo physiologic changes inside the vector.
Trend- Movement or change in frequency over time, usually upwards or downwards.
Trend, secular- Changes over a long period of time, generally years or decades.
Trial, clinical- An experimental study using data from individual animals. Investigators identify the type of exposure that each animal has had and then follow the animals' health status to determine the effects of the exposure.
Trial, randomized clinical- A clinical trial in which animals are randomly assigned to exposure or treatment groups.

U, V, W, X, Y, Z
Validity- The degree of accuracy of a measurement. For survey instruments, validity refers to what the questions actually measure in practice, as compared with what they are intended to measure.
Variable- Any characteristic or attribute that can be measured and can have different values.
Variable, continuous- A variable that has the potential for having an infinite number of values along a continuum. Common examples are height and weight.
Variable, dependent- In a statistical analysis, a variable whose values are a function of other variable.
Variable (or data), discrete- A variable that is limited to a finite number of values; data for such a variable.
Variable, independent- An exposure, risk factor, or other characteristic being observed or measured that is hypothesized to influence an event or manifestation (the dependent variable).

Variance- A measure of the dispersion shown by a set of observations, defined by the sum of the squares of deviations from the mean, divided by the number of degrees of freedom in the set of observations.
Vector- In epidemiology, an animate intermediary in the indirect transmission of an agent that carries the agent from a reservoir to a susceptible host.
Vehicle- In epidemiology, an inanimate intermediary in the indirect transmission of an agent that carries the agent from a reservoir to a susceptible host.
Virulence-The measure of severity of a disease, expressed as the proportion of animals with the disease and become extremely ill or dies.
Zoonoses- An infectious disease that is transmissible from animals to humans and vice versa.

Suggested Readings

1. Dicker, R.C., Coronado, F., Koo, D. and Parrish, R.G., (2006). Principles of Epidemiology in Public Health Practice, 3rd Edition. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, Office of Workforce and Career Development, USA.
2. OIE – Terrestrial Animal Health Code (2011).

4. Types of Epidemiological Studies for Livestock Disease Investigation

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In the light of population explosion, urbanization with decrease in the land availability for agriculture, and need for food with quality nutrition especially the protein, Animal husbandry is gaining importance worldwide. The era of back yard farming has shifted and farmer is thinking it as an capital investment in farming sector and expecting a good profit. This calls for good management practices and healthy livestock. The livestock disease prevention and control is the foremost agenda for any country to prosper in livestock segment. To achieve this, an early diagnosis of the disease in the population is necessary. In other words, study of disease in the population, Epidemiology, is the need of the hour. Epidemiology has been defined by many earlier workers but the definition given by Center for Disease control and Prevention (CDC) is exhaustive. The CDC has defined epidemiology as the study of the distribution and determinants of health-related states or events in specified populations, and the application of this study to the control of health problems. Some workers have defined epidemiology as the frequency of distribution and determinants of disease in population used for control of animal health problems. The epidemiological study will also include surveillance, monitoring and disease diagnosis. With the inflexible law governing the import and export of livestock and livestock products, there is a requirement of national surveillance programme to record the occurrence or non-existence of a particular disease. Thus a systematic epidemiological study is necessary for livestock disease investigation is required to enhance the acceptability of livestock and livestock products in the international market. The objective of an epidemiological research is to garner valid and precise information about the causes, prevention and treatments for disease. Epidemiologic research encompasses several types of study designs, including experimental studies and observational studies such as cohort and case–control studies. Each type of epidemiologic study design simply represents a different way of gathering and collating information. The selection of one design over another depends on the particular research objectives, concerns about validity and efficiency, and practical and ethical considerations. One of the methods for getting an answer to a research question is to conduct an experiment. But in majority of cases, it is not feasible to conduct the experiments due to various scientific reasons, high costs, and thorny ethical issues. Hence, observational studies are generally conducted. Observational studies are considered "natural" experiments because the investigator lets nature take its course. These studies provide information on exposures that occur in natural settings, and they are not limited to preventions and treatments. Furthermore, they do not suffer from the ethical and feasibility issues of experimental studies. An attempt is made in this paper to enumerate the various epidemiological studies used for livestock disease investigation. The term study/investigation includes both surveillance, which is to monitor aspects of disease occurrence and spread that are pertinent to effective control, and the epidemiological research, the main objective of which is to harvest valid and precise information about the causes, preventions, and treatments for disease.

The experimental studies, also known as trials, investigate the role of one or more factors or agents in causing/preventing/control of disease. Because experimental studies closely resemble controlled laboratory investigations, they are thought to produce the most scientifically rigorous data of all the designs. However, experimental studies are often

infeasible because of difficulties enrolling participants, high costs, and thorny ethical issues, most epidemiologic research is conducted using observational studies.

The two principal types of observational studies are cohort and case–control studies. A classical cohort study examines one or more health effects of exposure to a single agent. Subjects are defined according to their exposure status and followed over time to determine the incidence of health outcomes. In contrast, a classical case–control study examines a single disease in relation to exposure to one or more agents. Cases are those which have the disease of interest and controls are those which are a sample of the population that are part of study but not having disease. The purpose of the control group is to provide information on the exposure distribution in the population that gave rise to the cases. Investigators obtain and compare exposure histories of cases as well as controls.

Some researchers use additional observational study designs such as cross-sectional studies and ecologic studies. A cross-sectional study examines the relationship between a disease and an exposure among individuals in a defined population at a point in time. Thus, it takes a snapshot of a population and measures the exposure prevalence in relation to the disease prevalence. An ecologic study evaluates an association using the population rather than the individual as the unit of analysis. The rates of disease are examined in relation to factors described on the population level[11]. Table 1 summarizes the types of epidemiological studies used for livestock disease investigations.

Table 1: Types of Epidemiological studies for livestock disease investigations*

Type of study	**Features**
Experimental study design	Studies preventions and treatments for diseases; investigator actively manipulates which groups receive the agent or treatment under study
Observational	Studies causes, preventions, and treatments for diseases; investigator passively observes as nature takes its course.
Cohort	Typically examines multiple health effects of an exposure; animals are defined according to their exposure levels and followed for disease occurrence.
Case–control	Typically examines multiple exposures in relation to a disease; animals are defined as cases and controls, and exposure histories are compared.
Cross-sectional	Examines relationship between exposure and disease prevalence in a defined population at a single point in time.
Ecological	Examines relationship between exposure and disease with population-level rather than individual-level data.

(Adopted from Principles of Epidemiology in Public Health Practice, 3rd Edition, CDC[10])

The aim of disease investigation is to determine the relationship between an exposure and a disease with validity and precision using a minimum of resources. Validity is defined as the lack of bias and confounding. Bias is an error committed by the investigator in the design or conduct of a study that leads to a false association between the exposure and disease. Confounding reflects the fact that epidemiologic investigation is conducted among free-living animals with unevenly distributed characteristics. As a result, epidemiological studies that try to determine the relationship between an exposure and disease are susceptible to the disturbing influences of extraneous factors known as confounders. Precision is the lack of

random error, which leads to a false association between the exposure and disease just by "chance," an uncontrollable force that seems to have no assignable cause.

Experimental Studies

Experimental studies are conducted to understand about the prevention or treatment of a disease especially when a high degree validated data is necessary to draw an inference. Obtaining such data is not possible in an observational study. The high degree of validity in an experimental study arises mainly from investigators' ability to randomize subjects to either the treatment group or the comparison group and thereby control for distortions produced by confounding variables. A high level of validity may be needed for studying a prevention or treatment that is expected to have a small effect—usually defined as a difference of 20% or less between groups. A difference of this size is difficult to detect using an observational study because of uncontrolled bias and confounding. When the difference between groups is small, even a small degree of bias or confounding can create or mask an effect. Although most scientists agree that well-conducted experimental studies produce more scientifically rigorous data than do observational studies, several tricky issues make it difficult to conduct experimental studies. These issues include noncompliance, the need to maintain high follow-up rates, high costs, and numerous ethical issues. All such issues are to be addressed when considering this design.

Observational Studies or Analytical studies

Analytical studies can provide the necessary information to help evaluate the causality of an association and estimate the magnitude of risk. For each animal included in the study, baseline information is obtained about their disease status, their exposure to various contaminants and confounding characteristics. Analytical studies are either longitudinal or cross-sectional. In a longitudinal study, the time sequence can be inferred between exposure and disease; in other words, exposure precedes disease. In a cross-sectional study, exposure and disease information relate to the same time period; in these studies, it may not always be correct to presume that exposure preceded disease. The cross-sectional study design was used to investigate possible risks of *Peste des Petits Ruminants* disease in goats and reproductive and developmental risks.

Observational studies can be used to study the effects of a wider range of exposures than the experimental studies, including preventions, treatments, and possible causes of disease. For example, observational studies provide information to explain the causes of disease incidence and the determinants of disease progression, to predict the future livestock health care needs of a population, and to control disease by studying ways to prevent disease and prolong life with disease. The main limitation of observational studies is investigators' inability to have complete control over disturbing influences or extraneous factors. Once an investigator has decided to conduct an observational study, the next decision is usually whether to select a cohort or case–control design. Because a cohort study can provide information on a large number of possible livestock health effects, this type of study is preferable when little is known about the health consequences of an exposure. A cohort study is also efficient for investigating a rare exposure, which is usually defined as a frequency of less than 20%. Case–control studies are preferable when the knowledge on the etiology of a disease is limited since such studies can provide information on multiplicity of possible risk factors. Case–control studies take less time and cost less money than cohort studies, primarily because the control group is a sample of the source population. Secondly, they are more efficient than cohort studies for studying rare diseases because fewer animals are needed. Because of their relatively smaller sample size, case–control studies are preferred when the

exposure data are difficult or expensive to obtain. Further, these studies are desirable when the population is dynamic and constantly changing.

Case-control studies have a few important disadvantages. First, because of the retrospective nature of the data collection, there is a greater chance of bias. Hence, one school of thought is that case–control studies are not well suited for detecting weak associations. The second disadvantage is difficulty in establishing the correct temporal relationship between exposure and disease since the data is retrospective.

Before undertaking a cohort study, it is imperative to decide whether it is a retrospective or prospective study taking into consideration the practical constraints of time and money, and the availability of suitable study populations and records. In making this decision, the investigator must also take into account the complementary advantages and disadvantages of retrospective and prospective cohort studies. For example, a retrospective design must be used to study historical exposures, retrospective cohort studies are more efficient than prospective ones for studying diseases with long induction and latent periods. However, minimal information is usually available on the exposure, outcome, confounders, and contacts for follow-up because retrospective cohort studies typically rely on existing records that were not designed for research purposes. In addition, the use of retrospective data makes it more difficult to establish the correct temporal relationship between the exposure and disease.

In prospective cohort studies, investigators can usually obtain more detailed information on exposures and confounders because they have more control of the data collection process and can gather information directly from the farmer or by examining the animal (patient) itself. Follow-up may be easier because the investigator can trace the information from animals/farmer by maintaining periodic contact with the farmer / animals. Prospective cohort studies are considered less vulnerable to bias than retrospective studies, because the outcomes have not occurred when the cohort is assembled and the exposures are assessed. In addition, it is easier for investigators to establish a clear temporal relationship between exposure and outcome.

Cross–Sectional and ecological studies

A cross-sectional study examines the relationship between diseases (or other health-related characteristics) and other variables of interest as they exist in a defined population at one particular time. Unlike populations studied in cohort and case–control studies, in cross-sectional study the populations are commonly selected without regard to exposure or disease status. Cross-sectional studies take a snapshot of a population at a single point in time and hence measure the exposure prevalence in relation to the disease prevalence. In other words, current disease status is examined in relation to current exposure level. The main disadvantage of cross-sectional study is, it cannot infer the temporal sequence between exposure and disease if exposure is a changeable characteristic. Ecological studies examines the rates of disease in relation to a population level factor. The Population level-factors include summaries of individual population members or livestock units, environmental measures and global measures. In these studies, the study groups are identified by place, time, or a combination of the two. Ecological fallacy and lack of information on important variables are the limitations of ecological studies.

Random and Systematic Error

Biases that occur during the design and conduct of a study can lead to a false or spurious association or a measure of risk that departs systematically from the true value. All reported epidemiological associations require evaluation of random and systematic error so that results can be interpreted properly. Systematic error (bias) affects the validity of a study's observed association; random error affects the precision of the estimated magnitude of the risk. Random error is governed by chance and is influenced by the size of the study. The likelihood that a positive association is due to random error can be assessed by calculating the level of statistical significance ("P" value) or confidence interval (CI). A small P value (p) or a CI that does not include unity (1.0) suggests that chance may be an unlikely explanation for an observed association.

Potential sources of systematic error include observation, selection, misclassification and confounding biases. When information on exposure and disease are collected by methods that are not comparable for each livestock unit (e.g., selective recall), an incorrect association will be due to observation bias. When the criteria used to enroll livestock units in the study are not comparable, the observed association between exposure and disease will be due to selection bias. A wrong diagnosis of disease or assessment of exposure can result in misclassification bias. This type of bias may be randomly distributed (non-differential misclassification bias), which almost always biases study results towards the direction of not observing an effect (or observing a smaller change in risk than may actually be present), or it may be non-random (differential misclassification bias), which can result in either higher or lower estimates of risk, depending on how the misclassification is distributed.

Confounding bias may convey the appearance of an association *i.e.,* a confounding characteristic rather than the putative cause or exposure may be responsible for all or much of the observed association. Although negative confounding bias may occur, concern is usually with positive effects of confounding bias-is confounding bias responsible for the observed association? Confounding bias is generally present in all epidemiological studies and should always be evaluated as a possible explanation for an association[16]. Information on known or suspected confounding characteristics is collected to evaluate and control confounding during the analysis. In the design of case-control studies, matching is a technique that is used to prevent confounding bias. Techniques are also available to assess and control confounding during the data analysis. In an experimental epidemiological study, randomization is possible; that is, each individual in the study has an equal or random chance of being assigned to an exposed or unexposed group. Because of this random assignment of exposure, all characteristics, confounding or not, tend to be distributed equally between the selected study groups of different exposure.

Procedures in the study's design and conduct are used to prevent or reduce possible bias. If bias has been identified in a study, the direction of the bias can often be determined, but its effect on the magnitude of the association may not. For example, information may be available to determine whether the bias was responsible for an increased or decreased likelihood of observing an association, but its magnitude usually cannot be estimated.

Odds Ratio and Risk ratio

Two basic measures of an association between exposure and disease in analytical studies are the rate ratio or relative risk (RR) and exposure odds ratio (OR). An RR or OR of 1.0 indicates no association; any other ratio signifies either a positive or negative association. For example, an RR or OR of 1.5 indicates a 50% increased risk among the exposed. Decreased risk and protective effects are indicated by an RR or OR that is less than 1.0.The size of the

relative risk and odds ratio is also used to help assess if an observed association may be spurious. An RR or OR of 0.9–1.2 indicates essentially no association. Associations in this range are generally considered too weak to be detected by epidemiological methods. It is difficult to interpret a weak association. One or more confounding characteristics can easily lead to a weak association between exposure and disease, and it is usually not possible to identify, measure or control weak confounding bias. On the other hand, a large relative risk is unlikely to be completely explained by some uncontrolled or unidentified confounding characteristic. When the study has a reasonably large number of animals and the relative risk or odds ratio is large, random variability and confounding bias are much less likely to be responsible for an observed association. Table 2 shows the criterion for strength of association in an epidemiological study.

Table 2: Criteria for strength of association of an epidemiological study

Odds ratio or Risk ratio	Strength of association
1.0	None
1.0-1.5	Weak
1.5-3.0	Moderate
3.1-10.0	Strong
>10.0	Infinite

Risk difference and Attributable Risk

The basic approach for identifying risk factors is to compare the frequency of disease among groups with and without the risk factor or exposure under consideration. Because groups vary in size, rather than use the number of cases of disease, epidemiologists estimate the proportion of each population that has the disease in question. To further standardize the comparison, we usually stipulate a period of observation, such as a year. The resulting number new cases diagnosed per number of animals being observed is known as the rate of disease. For example, the rate of PPR in India is approximately 0.0001 per year, shorthand for one case per 10,000 animals per year (NIVEDI, 2014). The Rate difference or Attributable risk is the difference obtained by subtracting the rate of disease in the population without the risk factor from the rate of disease in the exposed population. We attribute this excess of disease to the risk factor(s).

Population Attributable Risk

The Population Attributable Risk (or Population Attributable Fraction) indicates the number (or proportion) of cases that disease would not occur in a population if the causal factors were eliminated. The attributable risk in a population depends on the prevalence of the risk factor and the strength of its association (relative risk) with the disease.

Causality of an epidemiological association

Epidemiological associations may be causal; however, before causality can be assessed, each study must be evaluated to determine whether its design is appropriate, the study size is adequate and systematic bias has not influenced the observed association. In addition, the association should be consistent with prior hypotheses and previous study results, and its magnitude should be moderately large. Causality requires sufficient evidence from several well designed and well conducted epidemiological studies in various geographic areas. Supporting data obtained from other studies are also important. Guidelines are available to help epidemiologists assess the possible causality of associations observed in well designed and well conducted studies. Epidemiological data should be interpreted with caution and in

the context of other available scientific information. Epidemiologists apply the following guidelines to assess evidence about causality.

1. Biological plausibility. When the association is supported by evidence from clinical research or toxicology about biological behavior or mechanisms, an inference of causality is strengthened.
2. Temporal association. Exposure must precede the disease, and in most epidemiological studies this can be inferred. When exposure and disease are measured simultaneously, it is possible that exposure has been modified by the presence of disease.
3. Study precision and validity. Individual studies that provide evidence of an association are well designed with an adequate number of study samples (good precision) and well conducted with valid results (i.e., the association is not likely due to systematic bias).
4. Strength of association. The larger the relative risk or odds ratio, the less likely the association is to be spurious or due to unidentified confounding. However, a causal association cannot be ruled out simply because a weak association is observed.
5. Consistency. Repeated observation of an association under different study conditions supports an inference of causality, but the absence of consistency does not rule out causality.
6. Specificity. A putative cause or exposure leads to a specific effect. The presence of specificity argues for causality, but its absence does not rule it out.
7. Dose–response relationship. A causal interpretation is more plausible when an epidemiological gradient is found (e.g., higher risk is associated with larger exposures).
8. Reversibility or preventability. An observed association leads to some preventive action, and removal of the possible cause leads to a reduction of disease or risk of disease.

Epidemiology for Risk Assessment

When scientists perform risk analyses, the best source of information on specific contaminants health effects is data from epidemiologic studies. Epidemiologists analyze how health-related events are distributed in specific animal populations—who gets sick with what illnesses, when, and where. By comparing groups with different illness rates and looking at demographic, genetic, environmental, and other differences among these groups, epidemiologists seek to determine how and why certain groups get sick. These studies are designed to inform public health policies and help prevent further harm. Epidemiologists may consider many possible determinants to explain patterns of illness, including physical, biological, social, cultural, and behavioral factors. In each case they seek to explain associations between certain exposures, risk factors or events, and illnesses or outcomes

To explore these associations, analysts have two basic study design options. Cohort studies follow a group of individuals who share some common characteristic such as age, place of residence, or exposure to a hazard, and study the frequency of illness in this group to see how strongly certain risk factors are associated with becoming sick. Researchers may also follow a control group that does not share the common factor with the cohort that is the study's subject. Whether they involve one group or two, cohort studies start with exposures and follow subject through time to find the outcomes. In contrast, case-control studies enroll a group of people who already have the disease of interest (the case group) and a group of animals who do not have the disease but match the case group members as closely as possible in other ways (the control group). Researchers then work backwards to identify risk factors

that may have caused the case group to get sick, and compare the groups to test how strongly these risk factors are associated with illness. Case-control studies start with the outcome and look backward to explain its causes.

To conclude, it is necessary that a researcher takes into consideration all aspects before designing the study so that it is bias free, statistically validated and scientifically accepted.

Suggested Readings

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5. Current Status of Veterinary Epidemiology in India

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The World Organisation for Animal Health (commonly referred to by the acronym of its original French name Office International des Epizooties [OIE]) was created in 1924 with the aim of controlling the international spread of infectious animal diseases. In this context the OIE has stressed that the teaching of fundamental scientific disciplines - virology, bacteriology, parasitology, epidemiology, risk analysis, immunology and vaccinology are all vital components in all veterinary education programmes and very important issues with respect to prevent and control animal diseases, outbreak investigations, spread of animal diseases including zoonoses. These are important fields of activities in veterinary epidemiology and the application of modern epidemiological methods now becomes a precondition for successful disease control.

From the Past, Emerges the Present

Some of the fundamentals of epidemiology have been the basis for global applications in for infectious disease investigations, confirmation and control. These fundamentals provide insights into the understanding of disease processes, progress and outcome which epidemiology attempts to correlate for immediate responses. Let us proceed to recollect some of them. Emerging diseases in man and animals coupled with increasing international trade has prompted an increased demand for veterinary surveillance systems which demands a structured approach to determine priorities for surveillance and subsequently a method to develop risk profiles for animal diseases for which some of the basic tools of epidemiology assist. The starting point for an epidemiological study is a certain characteristic or exposure rather than a disease which is recording of an observation.

Epidemiological observations

This depends on the quality of the data which is commonly described with the use of the following four terms:

- Accuracy: Accuracy is the degree to which a measurement represents the true value of the attribute.
- Precision: Precision is the reproducibility of a study result, that is, the degree of resemblance among study results.
- Reliability: Reliability is a measure of how dependable an observation is exactly the same when repeated.
- Validity: Validity is the extent to which the study measures what it is intended to measure.

Diagnosis of disease

Before a disease can be studied, it is necessary to define it and the variables that are the basis for such diagnosis may depend upon:

- Subjective observations by the owner/patient (symptoms)
- Subjective observations by the clinician/investigator (signs)
- Objective observations (post mortem lesions/diagnostic tests)

Diagnostic accuracy can vary from one disease to another and also between one group of individuals and another. This diagnostic accuracy is described by the following

- "Sensitivity" refers to the probability that the test result is positive, given that the population tested actually have the disease that the test is supposed to detect.
- "Specificity" to the probability that the test result is negative, given that the population tested does not have the disease that the test is supposed to detect.
- "Positive predictive value" refers to the probability that the population having the disease tested will have positive test result.
- "Negative predictive value" refers to the probability that the actual disease state is negative given that the test result is negative.

Epidemiological measures of disease

Measuring diseases are central to all epidemiological activity. Such measures can be formulated in a variety of ways:

- absolute numbers
- numbers related to size of the population
- incidence and prevalence

Incidence describes the frequency of occurrence of new cases *during a time period* while prevalence describes the proportion of the population which has the disease *at one specific point in time*.

Prevalence is dependent on the incidence and the duration of disease. In a stable situation this may be expressed as follows, $P/(1 - P) = I \times D$ where D indicates average duration of the disease and I the infectiousness. The measures of disease occurrence may be calculated for a whole population or calculated for parts of the population wherein the former is called "crude" measure and in the latter "specific" measure. Specific rates provide the most detailed information about the pattern of the disease in a population.

Measures of association

In epidemiological studies the frequency of disease among individuals with a certain characteristic are generally compared with the corresponding frequency among those which do not have that characteristic and the compared groups are referred to as "exposed" and "non- exposed".

An exposure or attribute that increases the probability of a disease is a risk factor and epidemiologists calculate the measure of the association to estimate the risk of developing a disease and an exposure.

The most frequently used measures of association are the *attributable risk (AR), relative risk (RR)* and the *odds ratio (OR).*

Attributable risk is the difference between the incidence of the disease in the exposed group (Ie) and incidence of the disease in non-exposed group (I_0)

$AR = Ie - I_0$

Relative risk (RR) indicates the average risk of disease due to a given exposure in the exposed group and it is the ratio of the incidence of the disease in exposed group (Ie) divided by the corresponding incidence of the disease in the non-exposed group (I_0).

$RR = Ie/ I_0$

A relative risk of 1.0 indicates that the incidence rates of disease in the exposed and nonexposed groups are identical and thus that there is no association observed between the exposed and the diseased. A value greater than 1.0 indicates an increased risk among those exposed to the factor. A relative risk less than 1.0 means that there is an inverse association or a decreased risk amongst those exposed.

Designing strategies in epidemiological research

The basic design strategies used in epidemiological research focusses on describing the distributions of disease (*descriptive epidemiology*) or elucidating its determinants (*analytical epidemiology*). Descriptive epidemiology is concerned with describing the general characteristics in relation to person, *place in time*. Descriptive data provide valuable information to plan effective prevention or education programs and are primarily useful for the formulation of hypotheses that can be tested subsequently using an analytical design.

Statistical Inference

Statistical inference is part of epidemiology because the observations studied by epidemiologists are subject to random fluctuations and the testing of hypotheses is a statistical procedure to determine the probability that the data collected are consistent with the specific hypothesis under investigation.

From the present to the future of Epidemiology

Information technology (IT) is computer-based techniques for data manipulation, storage, dissemination, publication, and retrieval of epidemiological methods through computer-based problem sets, case workups, outbreak investigations, and tutorials. The goal is to improve teaching and research in epidemiology worldwide. The concepts, methods, and principles can easily be applied to veterinary medicine. The Association for Veterinary Epidemiology and Preventive Medicine (AVEPM) seeks to increase awareness of issues in veterinary epidemiology. The AVEPM Web site (http://www.cvm.uiuc.edu/avepm/) includes a listing of educational software and Web sites supporting epidemiology and public health education.

Disease mapping and risk assessment in veterinary epidemiology

Disease mapping and risk assessment are important tasks in the area of medical and veterinary epidemiology. The development of methods for mapping diseases has progressed considerably in recent years. Geographical Information Systems (GIS), Remote Sensing (RS), and Spatial Analysis represent new tools for the study of epidemiology, and their application has become more and more advanced, in particular to study the spatial and temporal patterns of diseases

Mathematical modelling

This now plays a key role in policy making, control-programme evaluation and monitoring of surveillance data. Developing models for livestock epidemics is challenging and relies on many inbuilt assumptions. Even when data are available, adapting existing models can be problematic. International collaborations are important particularly in data-poor settings for developing model-guided surveillance methods.

Conclusion

The objectives of this document, therefore was to provide for the basic issues in veterinary epidemiological applications and develop a framework which may hopefully lead to more uniform use and understanding for practical implementation. It is urgently necessary to intensify epidemiological concepts in applied research and practice

Epidemiology as we know is the study of the *distribution*, *dynamics* and *determinants* of disease *frequency* in populations. These three closely interrelated components encompass all epidemiological principles and methods where measurement of disease frequency quantifies the existence or occurrence of disease while the distribution of the disease considers *who* is getting disease and *where* and *when* the disease is occurring. Such information attempts to

describe patterns of the disease to promote efficient risk management and to facilitate risk communication, primarily in the context of formulating a hypothesis for possible causes and thereby preventive efforts. Let us therefore start to focus on epidemiology based approaches in animal health to ensure early detection of diseases and implementation of cost-effective remedies for corrective actions.

With infectious diseases frequently dominating news headlines, professionals, policy makers and infectious disease researchers, increasingly need to understand the epidemiological techniques both conventional and recent applications to be able to interpret and critically evaluate infectious diseases.

6. Sampling Techniques and Sample Size Estimation for Epidemiological Studies

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Sampling frame for sero prevalence of livestock disease in India is done by using SAS (9.3) software and using sampling technique of the method is probability proportional to size sampling (PPS). Another main part is in the making of sampling frame is the Epitool is very impotent, the Epitools (online software) is main role because of these utilities can be used to estimate sample sizes and to analyse results of 2-stage surveys for demonstrating disease freedom. For the first stage a sample of herds is selected while the second stage is to select a sample of animals from each selected herd. Current versions assume perfect test specificity but allow for imperfect sensitivity. Methods are also available for situations where a complete list of herd sizes is available and also where no information is available on the target population.

Sero surveillance

Sero surveillance complements disease surveillance. Disease notification data are necessary to detect outbreaks and to provide timely information on the patterns of disease. However, notification data may be biased due to misclassification of the disease and due to being incomplete. Sero surveillance measures immunity which can result from vaccination or past infection (including subclinical infection). Such information is particularly helpful for mathematical modelling to determine the potential for future epidemics, and potential age groups at risk. Sero surveillance can also be used to evaluate specific vaccination campaigns. We carry out sero surveillance for

- To identify vaccine preventable diseases
- Measurement of immunity in a population
- Sort of disease surveillance method

Sample procedure

In statistics and quantitative research methodology, a data sample is a set of data collected and/ or selected from a statistical population by a defined procedure. Typically, the population is very large, making a census or a complete enumeration of all the values in the population impractical or impossible. The sample usually represents a subset of manageable size. Samples are collected and statistics are calculated from the samples so that one can make inferences or extrapolations from the sample to the population. This process of collecting information from a sample is referred to as sampling. The data sample may be drawn from a population without replacement, in which case it is a subset of a population; or with replacement, in which case it is a multisubset. A complete sample is a set of objects from a parent population that includes ALL such objects that satisfy a set of well-defined selection criteria.

The best way to avoid a biased or unrepresentative sample is to select a random sample, also known as a probability sample. A random sample is defined as a sample where each individual member of the population has a known, non-zero chance of being selected as part of the sample. Several types of random samples are simple random samples, systematic samples, stratified random samples, and cluster random samples.

A sample that is not random is called a non-random sample or a non-probability sampling. Some examples of nonrandom samples are convenience samples, judgment samples, purposive samples, quota samples, snowball samples, and quadrature nodes in quasi-Monte Carlo methods.

Sample size determination is the act of choosing the number of observations or replicates to include in a statistical sample. The sample size is an important feature of any empirical study in which the goal is to make inferences about a population from a sample. In practice, the sample size used in a study is determined based on the expense of data collection, and the need to have sufficient statistical power. In complicated studies there may be several different sample sizes involved in the study: for example, in a survey sampling involving stratified sampling there would be different sample sizes for each population. In a census, data are collected on the entire population, hence the sample size is equal to the population size. In experimental design, where a study may be divided into different treatment groups, there may be different sample sizes for each group.
Sample sizes may be chosen in several different ways:

- expedience - For example, include those items readily available or convenient to collect. A choice of small sample sizes, though sometimes necessary, can result in wide confidence intervals or risks of errors in statistical hypothesis testing.
- using a target variance for an estimate to be derived from the sample eventually obtained
- using a target for the power of a statistical test to be applied once the sample is collected.

Larger sample sizes generally lead to increased precision when estimating unknown parameters. For example, if we wish to know the proportion of a certain species of fish that is infected with a pathogen, we would generally have a more accurate estimate of this proportion if we sampled and examined 200 rather than 100 fish. Several fundamental facts of mathematical statistics describe this phenomenon, including the law of large numbers and the central limit theorem.
In some situations, the increase in accuracy for larger sample sizes is minimal, or even non-existent. This can result from the presence of systematic errors or strong dependence in the data, or if the data follow a heavy-tailed distribution.
Sample sizes are judged based on the quality of the resulting estimates. For example, if a proportion is being estimated, one may wish to have the 95% confidence interval be less than 0.06 units wide. Alternatively, sample size may be assessed based on the power of a hypothesis test. For example, if we are comparing the support for a certain political candidate among women with the support for that candidate among men, we may wish to have 80% power to detect a difference in the support levels of 0.04 units.
The sampling process comprises several stages:

- Defining the population of concern
- Specifying a sampling frame, a set of items or events possible to measure
- Specifying a sampling method for selecting items or events from the frame
- Determining the sample size
- Implementing the sampling plan
- Sampling and data collecting
- Data which can be selected

Advantages of sampling method

- Reduce Cost. It is cheaper to collect data from a part of the whole population and is economically
  in advance.

- Great speed. Sampling gives more time in collection of data, so it is quickly and has a lot of time for collection of inflammation.
- Detailed Information. Investigator during studying a small universe provides a detail and comprehensive information's.
- Practical method. Sampling is the only practical method when the population is infinite.
- Much easier. it is much easier to collect information from many individuals in a universe.

Disadvantages of sampling method

- Careful sampling selection is difficult.
- Experts are required for careful study of the universe.
- If the information's is required for each and every unite in the study ,then it is difficult to interview each and every person in sampling method.

Sampling Frame

A survey may be a census of the universe (the study population) or may be conducted with a sample that represents the universe. Either a census or a sample survey requires a sampling frame. For a census, the frame will consist of a list of all the known units in the universe, and each unit will need to be surveyed. For a sample survey, the frame represents a list of the target population from which the sample is selected. Ideally it should contain all elements in the population, but oftentimes these frames do not. The quality of the sample and, to an extent, of the survey itself depends on the quality of the sampling frame. Selecting a sampling frame that is of high quality and appropriate both to the population being studied and to the data collection method is a key step in planning a survey. In statistics, a sampling frame is the source material or device from which a sample is drawn. It is a list of all those within a population who can be sampled, and may include individuals, households or institutions. Importance of the sampling frame-In many practical situations the frame is a matter of choice to the survey planner, and sometimes a critical one. Some very worthwhile investigations are not undertaken at all because of the lack of an apparent frame; others, because of faulty frames, have ended in a disaster or in cloud of doubt.

An ideal sampling frame will have the following qualities:

- all units have a logical, numerical identifier
- all units can be found – their contact information, map location or other relevant information is present
- the frame is organized in a logical, systematic fashion
- the frame has additional information about the units that allow the use of more advanced sampling frames
- every element of the population of interest is present in the frame
- every element of the population is present *only once* in the frame
- no elements from outside the population of interest are present in the frame
- the data is 'up-to-date'

The methods used for collection of serum samples in sero-surveillance

In some cases the sample designer has access to an "auxiliary variable" or "size measure", believed to be correlated to the variable of interest, for each element in the population. These data can be used to improve accuracy in sample design. Another option is probability proportional to size ('PPS') sampling, in which the selection probability for each element is set to be proportional to its size measure, up to a maximum of 1. In a simple PPS design, these selection probabilities can then be used as the basis for Poisson sampling. However, this

has the drawback of variable sample size, and different portions of the population may still be over- or under-represented due to chance variation in selections.
Systematic sampling theory can be used to create a probability proportionate to size sample. This is done by treating each count within the size variable as a single sampling unit. Samples are then identified by selecting at even intervals among these counts within the size variable. This method is sometimes called PPS-sequential or monetary unit sampling in the case of audits or forensic sampling. The PPS approach can improve accuracy for a given sample size by concentrating sample on large elements that have the greatest impact on population estimates. PPS sampling is commonly used for surveys of businesses, where element size varies greatly and auxiliary information is often.

Probability proportion to size sampling:
First stage: PPS sampling → larger clusters have bigger probability of being sampled Second stage: Sampling exactly the same number of individuals per cluster → individuals in large clusters have smaller probability of being sampled
Overall: Second stage compensates first stage, so that each individual in the population has the same probability of being sampled
PPS steps are-

- Calculate the sample size for each strata.
- Separate population data into strata. The following steps will have to be applied for each strata.
- List the primary sampling units (Column A) and their population sizes (Column B). Each cluster has
  its own Cluster Population Size (a).
- Calculate the cumulative sum of the population sizes (Column C). The Total Population (b) will be the last figure in Column C.
- Determine the Number of Clusters (d) that will be sampled in each strata.
- Determine the Number of Individuals to be sampled from each cluster (c). In order to ensure that all individuals in the population have the same probability of selection irrespective of the size of their cluster, the same number of individuals has to be sampled from each cluster.
- Divide the total population by the number of clusters to be sampled, to get the Sampling Interval (SI).
- Choose a random number between 1 and the SI. This is the Random Start (RS). The first cluster to be sampled contains this cumulative population (Column C). [Excel command =rand()*SI]
- Calculate the following series: RS; RS + SI; RS + 2SI; …. RS+(d-1)*SI.
- The clusters selected are those for which the cumulative population (Column C) contains one of the serial numbers calculated in item 8. Depending on the population size of the cluster, it is possible that big clusters will be sampled more than once. Mark the sampled clusters in another column (Column D).
- Calculate for each of the sampled clusters the Probability of Each Cluster Being Sampled (Prob 1)
  (Column E).
  Prob 1= (a x d) ÷ b
  where, a= Cluster population
  b= Total Population
  d= Number of Clusters
- Calculate for each of the sampled clusters the Probability of each individual being sampled in each cluster (Prob 2) (Column G).

Prob 2= c / a

where, a= Cluster population

c= Number of individuals to be sampled in each cluster

- Calculate the overall basic weight of an individual being sampled in the population. The basic weight is the inverse of the probability of selection.

BW=1/(prob 1 * prob 2)

Random sampling is data collection in which every person in the population has a chance of being selected which is known in advance. Normally this is an equal chance of being selected. Random samples are always strongly preferred, as only random samples permit statistical inference.

Probability proportion to size is a sampling procedure under which the probability of a unit being selected is proportional to the size of the ultimate unit, giving larger clusters a greater probability of selection and smaller clusters a lower probability. In order to ensure that all units (ex. individuals) in the population have the same probability of selection irrespective of the size of their cluster, each of the hierarchical levels prior to the ultimate level has to be sampled according to the size of ultimate units it contains, but the same number of units has to be sampled from each cluster at the last hierarchical level. This method also facilitates planning for field work because a pre-determined number of individuals is interviewed in each unit selected, and staff can be allocated accordingly. It is most useful when the sampling units vary considerably in size because it assures that those in larger sites have the same probability of getting into the sample as those in smaller sites, and *vice-verse.*

Methodology

To prepare Sampling frame, steps as follows-

- First step is, in excel raw data of population data is arrange(like state wise, district wise, village wise and species wise) .Data is arrange by our convenient.
- Then data filter by like Goat, Sheep, Camel and etc.(raw data is filter by option of greater than or equal to 20 or 50 or 100 or depends on data)
- By method of (2) to create levels(level 1,level2 level3 and etc) using if condition in excel.
- After create the levels to find For the first stage a sample of herds is selected while the second stage is to select a sample of animals from each selected herd by using Epitools.

Epi Tools - 2-Stage surveys for demonstration of freedom: These utilities can be used to estimate sample sizes and to analyse results of 2-stage surveys for demonstrating disease freedom. For the first stage a sample of herds is selected while the second stage is to select a sample of animals from each selected herd. Current versions assume perfect test specificity but allow for imperfect sensitivity. Methods are also available for situations where a complete list of herd sizes is available and also where no information is available on the target population)

- In the Epi Tools first select 2-Stage surveys for demonstration of freedom then select Calculate sample sizes for 2-stage freedom survey with fixed herd/flock sensitivity and terminology as follows-

In this document some specific terminology relating to unit, cluster and population values have been used to try and simplify the formulae presented. These terms are explained here:

A unit is either an individual (animal, plant, etc) as part of a cluster, or a cluster as part of a larger population.

A cluster is a grouping level of individual units (animals, plants, fish, etc) at a higher level such as a herd, flock, tank, pen, farm, etc. Clusters usually are only considered at one level, but can occur at multiple levels (for example pens within farms within districts).
Unit sensitivity is the sensitivity at the unit level for a particular analysis. For cluster-level analyses, unit sensitivity is the sensitivity of the test (or combination of tests) used, whereas for population-level analyses unit sensitivity is the cluster-level sensitivity for clusters sampled.
Population sensitivity is a sensitivity calculated at some population or grouping level. Depending on the context, the population can be either a cluster of multiple individuals or a larger population comprising multiple clusters.
Component sensitivity is a population-level sensitivity, usually at a country or regional level, calculated for one part (component) of a surveillance system which comprises multiple separate components or activities.
System sensitivity is a population-level sensitivity, usually at a country or regional level, calculated from one or more components.

- By (5th method) using epitools the result will be number of herds to sample and maximum number of samples.
- All the above method import the excel data to SAS.
- Example of SAS programme for state wise sampling frame is as below-

```
   /* Programme for Karnataka Frame Work*/
libname nivedi 'C:\Users\admin\Desktop\sampleing\Samplingframe\nivedi';
run;
data AP;
set nivedi.sframe;
where State_Id=12;
run;
data ap;
set ap;
where cattle >50 and bufflaoe >10 and sheep >10 and Goat>11 and Pig>8;
run;
proc print data=ap;
run;
proc univariate data=ap;
        var Total_livestock;
        output out=percentiles1 pctlpts=33 67 pctlpre=P;
run;
proc print data=percentiles1;
run;


data ap;
set ap;
level=.;
if total_livestock<1156 then level=1;
if Total_livestock >=1156 and Total_livestock <2612 then level=2;
if Total_livestock>=2612 then level=3;
run;
```

```
proc sort data=ap out=ap;
key level;
run;

proc univariate data=ap;
        var Total_livestock;
        by level;
run;


ods rtf;
proc surveyselect data=ap method=pps n=(5 6 14) seed=4758 out=apframe;
size Total_livestock;
strata level;
run;


proc print data=apframe;
var district Tahsil Villages cattle bufflaoe sheep Goat Pig total_livestock level;
run;
ods rtf close;
```

Finally, to run the above programme to get output of the livestock data number of herds and number of samples to be made. And to get sample frame of each species SAS programme is as below -SAS programme for species wise:

```
ods rtf;
proc surveyselect data=KPS15.arp method=pps n=(8 12 30) seed=4758 out=arpframe;
size goat;
strata level;
run;
proc print data=arpframe;
run;
ods rtf close;
```

Example of Karnataka sampling frame, using above SAS programme is-
Karnataka sampling frame for sero-surveillance: (using 18th census livestock data)

	Karnataka Sampling frame			Village Livestock Population					Number of samples from each specieis to be collected						
Obs	District	Tahsil	Villages	Cattle	Buffaloes	sheep	Goat	Pig	Total livestock	Cattle	Buffaloes	Sheep	Goat	Pig	Total
1	Ramanagaram	US 0001	Gopahalli	172	24	276	125	33	648	7	1	11	5	1	25
2	Mandya	Maddur	Neelakantanahal i	80	156	259	129	65	721	3	6	9	5	2	25
3	Kolar	Chik Ballapur	Chillarpalli	165	33	507	28	9	779	6	1	17	1	0	25
4	Tumkur	Kunigal	Raghavana Hosur	85	50	615	237	128	1121	2	1	14	5	3	25
5	Koppal	Gangawati	Chikka Dankankal	597	94	224	121	31	1150	13	2	5	3	1	24
6	Bellary	Siruguppa	Nadivi	532	397	298	86	106	1466	9	7	5	2	2	25
7	Chikkaballapur	US 0001	Hampasandra	206	212	788	501	22	1898	3	3	11	7	0	24
8	Gulbarg	Shorapur	Bijaspur	521	210	305	952	61	2167	6	3	4	11	1	25
9	Bidar	Bhalki	Joldabka	617	418	486	242	218	2219	7	5	6	3	3	24
10	Koppal	Gangawati	Challur	551	1160	275	233	86	2349	6	13	3	3	1	26
11	Gulbarg	Yadgir	Yedhalli	1287	384	193	431	56	2464	14	4	2	5	1	26
12	Dharwad	Kundgol	Saunshi	944	413	698	393	105	2881	9	4	6	4	1	24
13	Gulbarg	Sedam	Handerki	1393	285	60	1071	13	2917	12	3	1	10	0	26
14	Bangalore	Bangalore North	Hesaraghatta	78	35	1217	1034	26	2984	1	0	11	9	0	21
15	Koppal	Koppal	Kawaloor	980	777	1152	481	61	3471	7	6	9	4	0	26
16	Tumkur	Chiknayakanhall i	Kenkere	1364	268	1564	527	49	3849	9	2	11	4	0	26
17	Gulbarg	Sedam	Mudhole	1357	370	1144	1447	87	4567	8	2	7	8	0	25
18	Bellary	Bellary	Sindhavalam	1188	597	2150	951	42	5078	6	3	11	5	0	25
19	Belgaum	Athni	Shedbal	1137	4083	540	938	352	7412	4	14	2	3	1	24
20	Chitradurga	Hiriyur	Rangenahalli	531	592	4856	1796	30	8022	2	2	16	6	0	26
21	Chitradurga	Molakalmuru	Nagasamudra	2379	461	2904	2916	65	9040	7	1	8	8	0	24
22	Gulbarg	Shorapur	Rajankollur	3773	2214	5228	51	37	11478	9	5	12	0	0	26
	Reserved villages														547
23	Belgaum	Gokak	Koujalgi	1537	1290	3833	2457	3074	12300	3	3	8	5	6	25
24	Belgaum	Chikodi	Benadi	139	2043	9280	496	40	12391	0	4	19	1	0	24
25	Bellary	Bellary	Badanahatti	1926	1274	24330	5243	60	33242	2	1	19	4	0	26
															75

7. Clinical Sample Collection and their Management

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Diagnosis of diseases is the corner stone for its proper eradication. Hence, the collection and shipment of appropriate samples to the laboratory have great importance. Collection of clinical samples is done primarily for four reasons:

1. Disease diagnosis
    a. *Direct examination (rapid diagnosis).*
    b. *Isolation of causative microorganisms.*
    c. *Serological and molecular investigations.*
2. Disease surveillance
3. Health certification
4. Monitoring the response to treatment and vaccination

Instructions for sample collection:

1. Collect blood samples from ailing, few in-contact and recovered animals.
2. Samples should be collected before the treatment is initiated.
3. All containers and instruments should be sterilized before collecting samples.
4. In case of deaths, necropsy should be performed and samples should be collected from the centre of the organs after singeing the surface with a hot spatula aseptically for isolation of the causative agent and histo-pathological (H.P.) examination.
5. Label all the specimen containers:

Species of animal……., Date of collection…….,
Preservative added if any……. Type of specimen……,

6. A brief history of the disease outbreak should accompany the sample as follows;

Town/village …….. , Species of animal ……...,
Other species infected if any……,
Population size……, Animal no…….,
No. animals affected……, Sporadic or epidemic……,
Vaccination/Treatment,if any……. Other information, if any……..

Procedures to be followed for despatch of clinical samples:

1. All sample containers should be labeled as "Pathological Specimen" "Fragile, handle with Care"
2. Biosafety measures (protective clothing, mask, gloves and laminar workstation) should be followed while collecting and despatching specimens from animals suspected for rabies, anthrax and other zoonotic diseases.
3. Whole blood may be despatched by mixing with an appropriate anticoagulant (EDTA, sodium citrate, etc.)
4. Serum samples should be despatched without adding any preservative. (Clotted blood without separating the serum should never be despatched)
5. Blood smears should be despatched after fixing with 70% alcohol.
6. The tissue specimens and the swabs for isolation of virus should be collected in 50% glycerine phosphate buffered saline or phosphate buffer solution containing 5% bovine serum albumin and antibiotic (e.g., gentamicin @ 50g/ml).

7. For isolation of bacteria, specimens should be collected in transport medium or in 1-2 ml normal saline solution and despatched to reach laboratory ON ICE at the earliest.
8. Organ samples should always be despatched in transport media or directly ON ICE as quickly as possible to prevent autolysis.
9. Urine samples for bacteriological study should be collected in sterile containers without any preservative and despatched within 1 hour.
10. If the samples cannot be examined, they should be refrigerated (but not frozen). If a refrigerator is not available, boric acid should be added to the urine at the rate of 0.5 gm/28ml suitable to preserve bacteria in urine and prevent their multiplication for up to 5 days.
11. Faecal samples should be despatched in screw cap metal or plastic containers preferably on ice.
12. Milk samples for bacteriological study should be despatched as quickly as possible. If the despatch is delayed, then they should be despatched by adding preservatives such as boric acid at the rate of 1 part of 5% boric acid to 10 parts of milk.
13. Cerebrospinal fluid, synovial fluid and fluids collected from the thoracic, abdominal or pericardial cavities should be submitted in sterile screw cap bottles without adding any preservative.

Sample collection methods

Blood: It is collected with a syringe containing any one of these anticoagulant such as Heparin (20 IU heparin/ml blood), Sodium Citrate (3.85%) 1ml/10ml of blood, EDTA 2% (1ml/10ml of blood) and Sodium oxalate 10% (1ml/100) ml of blood.

Serum: Blood is collected in a sterilized tube and is kept at 4^0 C to allow the formation of clot. When the clot retracts, supernatant is collected, centrifuged at 3,000 rpm for 15 min and decanted into fresh vial and stored.

Nasal swabs: The posterior nasal passages are most likely to contain viruses; hence nasal swabs moistened with transport medium should be collected. Similarly, for throat swabs, the tongue be pressed aside and the opposite posterior pharyngeal wall is rubbed around and collected with swabs moistened with transport medium.

Rectal and faecal samples: The swab is inserted without moistening the swab through the anal sphincter into the rectum and also faecal sample is collected directly from the rectum in a sterile vial.

Cerebrospinal fluid: The cerebrospinal fluid is withdrawn in the lumbosacral foramen between last lumber vertebra at the anterior and the sacrum at the posterior of cow. The site is shaved, washed with soap water, disinfected by 70% alcohol and collected with help of 18-gauge 2-inch needle and syringe.

Vesicular fluid: With the help of 1ml syringe fitted with a fine needle, the vesicular fluid is collected in a small quantity of transport medium.

Lesion scrapings: A sterile scalpel and forceps are used to collect the skin scrapings especially epithelial covering of the vesicle in to the transport medium.

General outline for the collection samples in different diseases:

A. 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	1. Blood smears from ear vein, swelling and discharges from natural orifices, peripheral blood, heart blood, spleen and swollen lymph nodes for

	of thorax and brisket region, muscular tremors, abdominal pain and sudden death followed by bloody discharges from natural orifices.	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.	Milk, for milk ring test and isolation. Serum sample (paired serum sample) for serological tests. Vaginal mucus, uterine fluid and blood on ice for isolation and PCR. Semen samples and swabs from the male reproductive organs for isolation and PCR. 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	1. 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 make a cracking sound under pressure.	Impression smears from the affected muscle and exudates from the swelling for demonstration of Causative organisms. Pieces of affected muscle and intestines on ice for isolation of organism.
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.).	Smears from contents of small intestine for demonstration of Gram positive rods with spores. 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	1. Blood and serum for dark field microscopic observation, isolation and PCR of leptospires. 2. Tissue from kidney, liver and

	inapparent condition and is characterized by sudden fever, muscle tremors, anorexia, haemoglobinuria, icterus and abortion.	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 (para TB)	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 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 *Actinobacillus mallei (Pseudomonas 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.

B. Viral diseases:

Name of the 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 Lyssavirus genus of the Rhabdoviridae family. Lyssa virus 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)	CSFV (previously called hog cholera virus) of the genus Pestivirus in the family Flaviviridae Flavivirus. 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

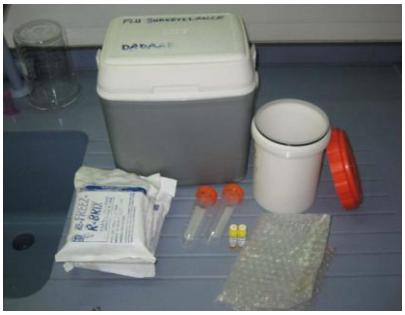
C. Parasitic Diseases

Theileriosis	Blood smears, Biopsy smears from swollen lymph nodes from early stage of disease fixed with Methanol
Babesiosis/ Anaplasmosis	Thin blood smears fixed in methanol.
Surra/Trypanosomiasis	Wet film examination of blood by hanging drop, fixed blood smears, blood in anticoagulant on ice.
Schistosomiasis	Dung sample (Avoid contact of water which may aid in hatching of the egg). Nasal schistosomiasis –Nasal discharge in normal saline, Nasal granuloma in normal saline.
Trichomoniasis	Vaginal or uterine discharges, prepucial scraping/ washing.
Gastro-Intestinal Parasitic Diseases	Faecal sample, affected internal organs in 10% formalin.
Ectoparasitic Infestations (Ringworm, Mange, Mites)	Deep skin scrapings in sterile vials.
External Fungal Infections	Skin scrapings in sterile vials

Storage of clinical samples

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

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.



Suggested Readings

- OIE Terrestrial Manual, 2009 by World organization for animal health.
- Sample collection procedure manual, diagnostic services of Manitoba Inc., USA.
- PD_ADMAS/Tech. Bull/8/2011 on Collection and dispatch of clinical samples for laboratory diagnosis of animal diseases.
- Rajeswari Shome, Nagalingam, M., Patil, S.S. and H. Rahman (2011) Collection and despatch of clinical samples for diagnosis of livestock. PD_ADMAS, Bangalore

8. Epidemiology and Control Programme of PPR in India

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India is a vast country with a population of 135.17 million goats and 65.06 million sheep (2012 19th Livestock census-DADF, GOI, http://dahd.nic.in). Small ruminants husbandry (sheep and goats) plays an important role in sustainable agriculture and employment generation and is primarily the work of small to marginal farmers and landless labourers. *Peste des petits ruminants* (PPR), otherwise called as '*Goat plaque*', is an acute, highly contagious, world organization for animal health (OIE) notifiable and economically important transboundary viral disease of sheep and goats associated with high morbidity and mortality. Clinically, the disease is characterized by high fever (pyrexia), oculo-nasal discharges, necrotizing and erosive stomatitis, enteritis, diarrhoea and bronchopneumonia followed by either death of the animal or recovery from the disease. The causative agent of the disease is a negative sense, single stranded-RNA virus, which belongs to the genus *Morbillivirus* of the family *Paramyxoviridae*. The PPR virus (PPRV) is genetically grouped into four lineages (I, II, III, and IV) based on the fusion (F) and Nucleocapsid (N) gene sequences analyses (Shaila et al., 1996; Dhar et al., 2002; Balamurugan et al., 2010). Because of the huge impact on production and PPRV emergences, it is considered as one of the main constraints in augmenting the productivity in small ruminants in enzootic countries like India.

In India, PPR was first reported from Arasur village, Villupuram district in Tamil Nadu during 1987 (Shaila, 1989). The disease was thought to be restricted in Southern India till severe epidemics swept through the rest of India in 1994 and after that it became enzootic in many northern states of India. Sequence and phylogenetic analyses of structural protein genes of different Indian isolates and vaccine strains of PPRV by different researchers, showed that all virus isolates /strains belong to lineage IV along with other Asian isolates since the disease was first reported in India (Shaila et al., 1996; Dhar et al., 2002; Balamurugan et al., 2010).

Despite strict control measures including statutory regulations along with availability of vaccines and diagnostics, this infection still remain a constant threat to sheep and goats. For the effective control and elimination of PPR, the strong support of accurate diagnostic methods or techniques or ELISA kits for mass screening and timely availability of vaccine and vaccination of the susceptible population are imperative once the epidemiology of the disease is fully understood. There is a need for a disease registry, both at state and at national levels, to ensure effective reporting and coordination of outbreak occurrence and monitoring. Therefore baseline epidemiological data is a prerequisite for a successful vaccination programme. PPR is still a poorly recognised disease, particularly with regard to epidemiological features such as transmission dynamics under different production systems. The clinical prevalence of PPR among sheep and goats could be of epidemiological significance, and data about PPR outbreaks, molecular and seroepidemiology of disease are essential and play an important role for effective disease control programme. Comprehensive information on epidemiology of PPR as a whole in India is not available except some few reports. Measuring the clinical prevalence of PPR in different geographical areas of the country with varying agro-climatic conditions may be helpful for establishing disease control measures/ strategies and can be useful for determining the actual infection rate. The analytical epidemiological study about incidence would be an extremely useful and provides sufficient

additional information about the disease, which are important in supporting control policy decisions. In this study, the epidemiological information about the status of outbreak or prevalence in sheep and goats in India has been analysed and discussed in details including the mortality, clinical- and seroprevalence, host susceptibility, pathozones, seasonal prevalence, occurrence, control strategies including on-going national control programme. The epidemiological data on PPR (from 1995 to 2014 from seventeen states in five zones of the country) in India including outbreaks data (monthly disease reports provided by different state animal husbandry departments and collaborating units of AICRP (All India Coordinated Research Project) on ADMAS) has been analysed using the disease records (most of the outbreaks have been diagnosed based on clinical signs at field by local veterinarians) available at National Animal Diseases Referral Expert System (NADRES) database in National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), Bengaluru (http://www.nadres.res.in).

The epidemiological information about the status of outbreak or prevalenec in sheep and goats, host susceptibility, the decadal, quinquennial and yearly temporal pattern, seasonal and species wise occurrence of PPR, PPR pathozones, endemicity, etc. in the different zones and states of the country has been analysed based on the cumulative outbreak reports and presented and depicted on the regional map of India using the EpiInfo™ software-2000 designed by the Centers for Disease Control and Prevention in the United States (http://www.cdc.gov/epiinfo) and using Mapinfo software (www.mapinfo.com). PPR is foremost important viral disease of small ruminants in India and is a major obstacle in augmenting productivity with trade restriction incurring great economic losses (Venkataramanan et al., 2005) and adversely affects the livelihood of marginal and small farmers as well as landless labourers as sheep and goats are reared in India by primarily the poor and economically downtrodden sectors of the farming community. As per rough estimate, the disease cause annual loss to the tune of 4000 million INR (DADF, GOI) in India.

In NADRES database of NIVEDI, PPR outbreaks reports are available since 1995 from seventeen states in five zones (North, South, West, East and Central) of the country. However, earlier to 1990, there are only two outbreak reports from Andhra Pradesh state during 1987 (Sudharshana et al., 1995), in addition to first Arasur 87 outbreak in India (Shaila et al., 1989). It was possible that the disease had existed before, until it was identified in some of these affected regions (Taylor et al., 2002). Indeed in the absence of proper diagnostics, the disease had often been misdiagnosed in favour of other pneumonic diseases such as pasteurellosis, contagious caprine pleuropneumonia (CCPP) and has precluded and delayed its recognition.An extensive clinical survey of PPRV infection has been difficult up to this point in time because the diagnostic tests that were available for either detection of PPRV or antibodies were not commonly implemented in different state diagnostic laboratories, possibly because the endemic nature of the disease was not known. It is still not clear whether the apparent geographical spread of the disease in the last two decades is real or reflects increased awareness, wider availability of diagnostic tools or even a change in the virulence of the virus. It seems most likely that combinations of these factors are responsible for the present knowledge of the disease distribution. Most of the states of India, have undertaken surveillance for prompt disease reporting of PPR as sensitive and specific diagnostic tools are currently available. Efficient mass screening diagnostic assays would be very useful for providing evidence indicating whether or not PPRV antigens are present in a susceptible population. Monoclonal antibody based competitive-ELISA and Sandwich ELISA for PPR antibody detection and antigen detection, respectively developed at Division

of Virology, IVRI, Mukteswar campus are the currently being employed assays for serosurveillance /monitoring and clinical prevalence throughout the country (Singh et al., 2004a,b).

Organized clinical surveys or confirmation of outbreak status has not been performed for PPR in India except for a few isolated clinical reports from different state Animal Husbandry Departments which included only regional data from various states of India since 1994.For example the laboratory confirmed 52 outbreaks during 1997 to 2003 and 69 outbreaks during 2003 to 2009 in a population of 211 million animals have been reported by different researchers (Singh et al., 2004c; Balamurugan et al., 2011; 2012b). On this basis it would not seem to be a very important disease, but it is not true in real sense. However, it is to correctly point out that many outbreaks are not reported properly and not laboratory confirmed, which will be a major problem for a successful control program. In India, several outbreaks go unrecorded due to inadequate animal disease reporting and surveillance systems. Plenty of PPR outbreaks have occurred in the past and are now occurring regularly throughout India. PPR is of increasing importance and likely to extend its geographic distribution especially in North Eastern states, as PPR outbreaks have been reported in Assam since 2010. Hence, it is extremely necessary to perform epidemiological surveys of this disease. Measuring the clinical prevalence of PPR in different geographical areas of the country with varying agro-climatic conditions may be helpful for establishing disease control strategies and can be useful for determining the actual infection rate.

The majority of PPR outbreaks have been diagnosed based on clinical signs at field level by local veterinarians. Based on these outbreak reports (from 1991 to 2014) in NADRES, PPR features among the top ten diseases reported in small ruminants. Among the viral diseases, PPR stands first and is the highest reported disease in sheep and goats. It is the major cause of mortality and accounts for 34 % of the mortalities reported in sheep and goats. A number of PPR outbreaks have been reported in different states of India with variable morbidity and mortality since 1994. Mortality in susceptible flocks varies from 10 to 100% and morbidity from 50% to 100%. However, this scenario is likely to change drastically once intensive vaccinations are carried in these target (sheep and goats) populations.

On analysis of PPR pathozones based on outbreaks reported showed a wide variation in the different states of the country. This could be due to disparities in sheep and goat husbandry practices and the agro-climatic conditions affecting the pattern of the natural vegetation which indirectly influence the socio-economic factors, the migration patterns of small ruminants in relation to season, flock size and the population intensity of the animals in the different states. Many districts of Andhra Pradesh, Karnataka, West Bengal, Himachal Pradesh, Jammu and Kashmir, Odisha and West Bengal fall under the high and moderate pathozone. PPR is widespread in the country with many low pathozone areas. Increased number of outbreaks have been reported in goats than in sheep in the different zones of the country, except in south zone where the number of outbreaks were greater in sheep. These findings may be correlated with variations in the sheep and goat husbandry practices within different geographic regions. The ratio of goats to sheep and the population intensity vary greatly under different agro-climatic conditions. PPR affects goats more than sheep and the population of goats to sheep is almost 2:1 in India. In case of south zone, the population of sheep is higher than goats. Highest number of outbreaks has been reported in south zone followed by east zone. Even though India is endemic to PPR, some states especially north-eastern states either free from disease or have very few reports. These states have a relatively small sheep and goat population and intermixing of these animals with the small ruminant

population from the rest of the country is usually limited because of very narrow connecting passage. In other way, the hilly terrain characterizing this region may further restrict the movement of animals and therefore the transmission of the virus between animals. Although south zone states like Karnataka and Andhra Pradesh have shown a decline in number of PPR outbreaks during the last 5 years 2009-2014 (probably due to vaccination), the disease is still reported regularly. States like West Bengal, Madhya Pradesh, Maharashtra and Rajasthan have reported an increasing trend of PPR occurrence during the last five years.

Temporal analysis of PPR reports showed a gradual increase in outbreaks since 1995. Highest numbers of outbreaks were reported between 2000 and 2007, after which there is a decline in PPR outbreaks. This could be due to availability of diagnostic assay during 2002 and implementation of vaccination programme in some states during 2005. Decadal trend of PPR outbreaks showed that increased number of districts was affected during 2004-2014 compared to the previous decade. Variation in the annual occurrence of the disease may be the result of various factors, including host, agent or environmental factors. Climatic factors favourable for the survival and spread of the virus may also contribute to the seasonal distribution of PPR outbreaks. Based on monthly data on PPR outbreaks, PPR has been found to occur throughout the year but was encountered most frequently during the lean period either in wet season/rainy season or during the cold dry season (December to February) (Hegde et al., 2009). Animal husbandry practices, agro climatic conditions, and geographical locations may have some effect on the seasonal distribution of the disease. This seasonal occurrence of the disease was correlated with the animal movements and climate factors in India. Most of the investigators have linked the PPR outbreak with introduction of new animals to the flocks.

The PPRV primarily affects goats and sheep and occasionally wildlife. However, no detailed study on the role of wild animals in the epidemiology of PPR was undertaken in India. Breed of the animal may also have effect on the outcome of PPR and its epidemiology. Occurrence of PPR associated with age of the animals has also been reported; young animals aged from 6 months to 1 year old are more susceptible than adult animals. Though PPR virus infects both sheep and goats, severity of the clinical symptoms are more predominant in goats than sheep. In most of the reports, goats were severely infected than sheep. Based on difference in the virulence of field strains for both species, sheep might have innate resistance to clinical development of the disease. Earlier report showed that, PPR can also be transmitted directly or indirectly from sheep or goats to cattle, providing a mechanism for the virus to survive outside of the environment in the unnatural host. PPRV needs close contact between infected and susceptible animals to spread because of either the labiality of the virus outside the host or low resistance of the virus in the environment. Significant quantities of virus are excreted in the secretions (the discharges from eyes, nose and mouth) of affected goats during the course of infection and are the important source of virus infection (Singh et al., 2004a). The infectious materials can also contaminate water, feed troughs and bedding, turning them into additional sources of infection. Trade of small ruminants at markets where animals from different sources are brought into close contact with one another affords increased opportunities for PPR transmission.

Cattle sero-converted following contact with the sick sheep and goats but did not develop any clinical signs. The PPRV may be adapted in cattle which are subclinically infected without showing any symptoms of illness under natural condition. The circulation of PPRV in unnatural host(s) might have a positive role or help in the control or restrict the spread of PPR in small ruminants in particular geographical area. This assumption may be due to the

possibility of adaption and change in virulence of the virus where small and large ruminants are reared in integrated farming systems (Balamurugan et al., 2012a). Seroprevalence studies of PPR in sheep, goats, cattle and buffaloes by different researchers also indicated an extensive endemicity of the disease in various states of India. The results suggested the natural transmission of PPRV infection among these animals under field condition. The prevalence of PPRV antibodies in sheep and goats indicated subclinical or in-apparent infection or non-lethal infections, which could be of epidemiological significance. It may reveal novel characteristics of the epidemiology and transmission of PPRV.

The role of PPR in the control of RP might have also helped to eradicate the disease due to the possibility of seroconversion of PPRV antibodies in cattle and buffaloes where small and large ruminants co-existed. In general, infected sheep and goats on subsequent recovery from the disease are protected from re-infection for their life. Recovery rates from PPRV infection are considerably lower for goats than for sheep, resulting in a low proportion of the goat population being protected from reinfection. On screening of a total of 318 serum samples from goats and sheep from 18 districts in five states (Meghalaya, Assam, Nagaland, Arunachal Pradesh and Manipur) of North Eastern India indicates that an overall seroprevalence of 11.63 % in goats. Similarly, on screening of random samples collected from different livestock (sheep, goats, cattle and buffaloes) species from five states, seropositivity seems to be 21.83%, which showed that under natural situation, this percentage of the animals are protected from reinfection (Balamurugan et al., 2014). Hence, the change in virus virulence ie., virus adaption in the bovines will also help to increase the recovery rates of the small ruminants, which in turn restrict the spread of disease in that particular area. These aspects needs confirmative study, based on the survival of the virus, host susceptibility, genetic mutation of the organism or change in the virulence of organism, change in the disease pattern or change in the exhibiting the prominent clinical signs etc., Control strategies may vary from country to country as per the prevalence of disease but in developing or under-developed countries the choices are limited. In India too, stamping out by slaughter is not feasible, both for economic and sentimental reasons. Social acceptance, public and regulatory support is essential for success of any disease control and eradication program. Society will readily accept the vaccination program. Hence, vaccination has become a recommended tool to support control and eradication efforts and limit the economic losses due to PPR (Singh et al., 2009; Singh, 2011). Therefore, PPR control and eradication depends mainly on rapid and accurate diagnosis, surveillance/monitoring and implementation of prompt vaccination programme.

The factors that favours the control and eradication of PPR include the capability of the country in the lines of RP eradication, restricted geographic distribution of the disease, transmission by droplet requiring relative close contact, short incubation period, no latency and economic incentive. Further, the availability of an effective vaccines (Sen et al., 2009), accurate mass screening diagnostic assays, an experienced infrastructure, expertise, success with eradication of RP under the NPRE, has provided the confidence and prompted us to propose a national level PPR control and eradication programme initially on the lines of NPRE and throughout the Asian continents later without much additional budgetary encumbrance. All the aforesaid elements required for a control programme are available (Singh et al., 2009, Singh 2011), which have further been recommended for a collaborative nation-wide program implemented by state Animal Husbandry Departments under the direction of the Department of Animal Husbandry, Dairying and Fisheries (DADF, GOI) and the co-operation of the local public under the guidance from the policy maker at the centre. Therefore, the launching of programme appears technically feasible, economically viable and

a practically attainable proposition. Hence, it has been decided by DADF, Government of India to undertake a National Control programme on PPR (NCP-PPR) in the 11th five-year plan (2007-12) with an aim to control and eradicate this disease from India in a time bound manner on the lines of rinderpest eradication. Accordingly, this proposed programme has been initiated by following eradication pathway of OIE during the year 2010-11 with a sum of INR 432.5 million in first phase. Vaccination strategies for the control of PPR would be slightly different from vaccination programmes for RP. A mass vaccination campaign to cover 80% herd or flock immunity would be needed to account for the population dynamics of sheep and goats, disparities in sheep and goats husbandry practices and the agro-climatic conditions affecting the pattern of disease (Singh 2011). The slaughtering of male goats at an early age combined with the high fecundity of the caprine species results in replacement of population (~30-40% naïve population appears) every year. Initially, in order to reduce economic losses due to PPRV, intensive vaccination of the entire population within a specified area would need to be undertaken. Subsequent vaccinations would then be performed on younger animals at approximately 6 months of age as suggested earlier (Singh 2011). The maternal antibodies in kids were detectable up to 6 months with a declining trend from the third month onwards and receded below the protective level by the fourth month in vaccinated and infected goats and PPR vaccination is recommended in kids, aged 4 months and born to immunized or exposed goats to avoid window of susceptibility in kids to PPRV and the effort to eliminate PPR infection from susceptible populations (Balamurugan et al., 2012a). The 4 to 6 months old young ones in and around the vaccinated flocks will be few not even >30 % of the population at that point of time. The vaccinated flocks available will be 70 % to 80% and provides the herd or flock immunity. Vaccinated animals, infected and recovered animals are protected from re-infection for the remainder of their lives.

In this direction, the strategies proposed in the control programme involving intensive vaccination of all susceptible sheep and goats and their three subsequent generations (approx. 30%) with 100% central assistance (http://www.dahd.nic.in). In the first phase of programme Kerala, Tamil Nadu, Karnataka, Andhra Pradesh, Maharashtra, Goa, Union Territories (UT) of Lakshadweep, Daman & Diu, Dadra & Nagar Haveli, Andaman & Nicobar Island and Puducherry were covered. In the past two years, the vaccination and sero-monitoring was carried out in the NCP-PPR in some states of India. The second phase has been taken up in the 12th five-year plan (2012-17), by the end of which the disease is expected to be fully controlled (http://www.dahd.nic.in). Research institutions will also be assisted for undertaking surveillance and monitoring of the disease during the surveillance stage. Available expertise especially scientific and technical manpower, trained scientific (veterinarians), technical and para-veterinary staff, in the country is being used for handling vaccines at various stages from production till delivery in the field (Singh et al., 2009). Professional commitment on the part of veterinarians and ancillary personnel involved in mass immunisation program is crucial to succeed vaccination programme. These prophylactic services would be gradually expanded by involving Public-private partnership (PPP) has especially participation of non-governmental organisations (NGOs) and cooperatives and private veterinary practioners in implementing and execution of disease control programs and therefore liaison with private vaccine manufacturers is also warranted. Finally, it is hoped that PPR in the direction of rinderpest will be eradicated in India within a decade or few more years. Overall, the present scenario of PPR in India warrants the studies to be undertaken with the objective to know the effect of agro climatic changes on the occurrence of PPR in small ruminants in different agro-climatic zones and to analyse the relationship of disease occurrence and risk factors to formulate modules for forecasting and forewarning. The

epidemiological information about the status of PPR outbreaks or prevalence in sheep and goats in India would be discussed during deliberation.

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9. Descriptive and Spatio-temporal Epidemiology

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Descriptive epidemiology

Descriptive epidemiology deals with the distribution of disease within a population and takes into account information regarding time (eg, year, season, day, hour), place (geographic variation--national, regional, local) and animal (eg, age, breed, sex, behaviours etc). Descriptive epidemiology provides a way of organizing and analyzing these data in order to understand variations in disease frequency geographically and over time, and how disease (or health) varies among animals based on a host of personal characteristics (person, place, and time). This makes it possible to identify trends in health and disease and also provides a means of planning resources for populations. In addition, descriptive epidemiology is important for generating hypotheses (possible explanations) about the determinants of health and disease. By generating hypotheses, descriptive epidemiology also provides the starting point for analytic epidemiology, which formally tests associations between potential determinants and health or disease outcomes.

Specific tasks of descriptive epidemiology:

- Monitoring and reporting on the health status and health related behaviors in populations
- Identifying emerging health problems
- Alerting to potential threats from bioterrorism
- Establishing public health priorities for a population
- Evaluating the effectiveness of intervention programs and
- Exploring potential associations between "risk factors" and health outcomes in order to generate hypotheses about the determinants of disease.

Types of Descriptive Epidemiology

1. Study of Ecology of the population
2. Livestock disease case reports/case series
3. Disease survey/ Cross sectional stuides

1. Study of Ecology: Ecological studies are studies of risk-modifying factors on health or other outcomes based on populations defined either geographically or temporally. Both risk-modifying factors and outcomes are averaged for the populations in each geographical or temporal unit and then compared using standard statistical methods. It helps in evaluating association of the factors determining the disease but no the causation of disease.

   Eg: Bluetongue and vector population and the temperature-humidity factors affecting the growth of the vector population.Transportation lorries played a major role in the transmission of CSFV before the primary outbreak was diagnosed in the major outbreak of CSF in Netherlands during 1997-98.
2. *Livestock Disease case reports/case series:* A case series (also known as a clinical series) is a type of medical research study that tracks subjects with a known exposure, such as patients who have received a similar treatment, or examines their medical records for exposure and outcome. Case series may be consecutive or non-consecutive, depending on whether all cases presenting to the reporting authors over a period were included, or only a selection. Case series have a descriptive study design; unlike studies that employ an

analytic design (e.g. cohort studies, case-control studies or randomized controlled trials), case series do not, in themselves, involve hypothesis testing to look for evidence of cause and effect. Case series are vulnerable to bias. For example, abortions in cows due to infectious agents wherein the samples are drawn from the same hospital wherein such cases are reported regularly. A case report is a detailed report of the symptoms, signs, diagnosis, treatment, and follow-up of an individual patient. Case reports may contain a demographic profile of the patient, but usually describe an unusual or novel occurrence. Some case reports also contain a literature review of other reported cases.

Most case reports are on one of six topics:

- An unexpected association between diseases or symptoms.
- An unexpected event in the course of observing or treating a patient.
- Findings that shed new light on the possible pathogenesis of a disease or an adverse effect.
- Unique or rare features of a disease.
- Unique therapeutic approaches.
- A positional or quantitative variation of the anatomical structures.
  E.g., *Haemorrhagic septicaemia* in a pig caused by extra intestinal pathogenic Escherichia coli (ExPEC) as a differential diagnosis in classical swine fever--case report and review of the literature

3. *Disease survey/ Cross sectional studies:* Surveys are used to determine the frequency of disease (prevalence) along with related factors at a point in time. If a survey collects information about exposure and disease status at the same time, this becomes a cross sectional analytic study. Estimate prevalence of disease of interest and populations with increased exposure (risk). Repeat surveys can be used to monitor changes in prevalence or exposures over time.
Eg: Seroprevalence of CSF in two states of NE India viz., Tripura and Mizoram. Cross sectional study of CSF in villages of Tripura and Mizoram bordering Bangladesh and Myanmar.
Summary: Descriptive and Analytic epidemiology are two important branches of epidemiology and sometimes used interchangeably. Terminologies used in this subject are to be clearly defined so that factors/determinants affecting the diseases can be associated logically.

Spatial Epidemiology:
Spatial epidemiology is the description and analysis of geographic variations in disease with respect to demographic, environmental, behavioral, socioeconomic, genetic, and infectious risk factors.

Importance of spatial epidemiology
It may be noted that many diseases are spatially constrained; for example, vector-borne and zoonotic diseases occur where and when vectors, animal hosts, pathogens and susceptible human populations overlap. Vectors, pathogens and animal populations are unevenly distributed in space and time and as a result risk for exposure to vectorborne diseases is spatially heterogeneous. Therefore, spatial models for the study and management of vector-borne disease risk have become common with the development of digitally encoded environmental data and computational tools such as geographical information systems (GIS).

It is estimated that over 90% of health data (animal and human) has a spatial or geographical component.

- Variations in disease outbreaks are better understood if neighbouring farm networks are taken into account?
- Geography can also be used as a proxy eg., measuring the distribution of outbreaks against vaccination activity, i.e is there more FMD in non-vaccinated areas
- Geography is a very useful framework for communication for example "how does the livestock health in Mizoram compare to Sikkim is more easily understandable when mapped.

Requirements for spatial analysis

1. Disease data: Time, number of cases (no. affected), total population, number of deaths
2. Demographic Data: Species wise data
3. Environmental Data: Rainfall, Temperature, Wind speed, Wind direction, Relative humidity, Vector Data
4. Socio-economic Data
5. Land Cover and Land Use Data
6. Soil Data
7. Digital Maps: Latitude, Longitude, Real time satellite images,
8. Finally, skill and will

What can be achieved in spatial epidemiology?

Disease mapping: Disease mapping is a field that concentrates on the spatial variation in the risk of disease. Basic map styles commonly used for disease mapping include Dot maps, Choropleth maps, Isopleth maps.

Dot maps, are suitable for presenting point data referenced in two-dimensional coordinate space, such as the locations of disease events.

Choropleth maps are tremendously common and useful. These use some existing system of boundaries (countries, states, counties, voting districts, etc.). In choropleth maps, data is grouped into or more levels or classes using slicing values. These maps show spatial variation of one or two variables at a time by using color, shades of grey and/or patterns.

Isopleth maps are especially well fitted for inspecting continuously varying phenomena, such as temperature, rainfall etc. These maps feature continuously varying color values basically by a line on a map that connects points of equal value.

Geographic correlation studies: These look at correlations between variables. For example, a study may investigate the occurrence of meningococcal infection. In doing this it might correlate the economical status and personal hygiene of people. One would expect that as level of infection to be more among economically weaker class. Some studies may focus on habitat mapping of a particular species of insect based on the data obtained from a small survey. Another example of geographical correlation studies is relating human taeniasis infection with heavily infected cysticercotic pigs.

The assessment of risk in relation to a point or line-source: Point and line-source studies assume a risk source that has the shape of a point or a line, such as a chimney or an electrical wire. Highly localized studies are conducted in order to discover possible increases in ill-health due to increased exposure from these specific types of sources.

Cluster detection and disease clustering: Cluster detection is carried out in order to detect raised levels of incidence of disease. If disease cases seem to form non-random patterns, that

is, clusters, then there is reason to suspect that the underlying effect is non-random. It can provide information on the etiological background of the disease. A spatial disease cluster may be defined as an area with an unusually elevated disease incidence rate. The identification of a cluster of disease can help epidemiologists determining putative environmental risk factors and lead to improved understanding of etiology.

What are the commonly encountered problems in spatial epidemiological studies?

- Non availability of good quality of data which may lead to Garbage in = Garbage out kind of situation.
- Georeferencing of attribute data
- Government data rich but information poor
- Lag time between environmental exposure and presentation
- Biological plausibility – does it make sense (critical thinking skills)
- Spatial data is expensive
- Spatial data must be accurate and up to date
- Complexity of analysis - requires training
- Complexity of activity – multiple exposures points
- Interpretation - ecological fallacy, aggregating data

Summary: Spatial epidemiology is the study of the spatial patterns, processes and determinants of health and disease. Spatial epidemiological methods use GIS and spatial based analytical tools.

10. Epidemiology of Brucellosis and Control Programme in India

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Brucellosis is highly contagious and economically important bacterial disease of livestock worldwide. It is most wide-spread zoonotic infection in the developed and developing world, transmissible from animals to humans. When brucellosis is detected in a herd, flock, region, or country, international veterinary regulations impose restrictions on animal movements and trade, which result in huge economic losses. These are the reasons why programs to control or eradicate brucellosis in cattle, small ruminants, and pigs have been implemented worldwide (OIE).

Brucellosis is caused by Gram-negative facultative intracellular bacteria of the genus *Brucella* either singly or by several *Brucella* species and biovars. On the basis of host preference, antigenic variation and biochemical characteristics, the *Brucella* genus is divided into terrestrial and marine species. The eight species which infect terrestrial mammals are *Brucella abortus, B. melitensis, B. suis, B. canis, B. ovis and B. neotomae* (Corbel and Brinley-Morgan, 1984). *B. microti* found to infect wild common voles and *B. inopinata* has been isolated from a human brucellosis case. A more recently two species namely *B. pinnipedialis* for strains infecting pinnipeds (seals, sea lions and walruses) and *B. ceti* from cetaceans (whales, porpoises and dolphins) were identified. Bovine brucellosis is usually caused by *B. abortus*, less frequently by *B. melitensis* and rarely by *B. suis*. In India, cattle and buffaloes harbor predominantly *B. abortus* biotype-1 followed by second most predominant biotype-3 and rarely other biotypes 1, 3, 6, 7 and 9.

Economic losses due to brucellosis

Despite decades of regulatory efforts worldwide, *Brucella* has resisted obliteration in livestock and wildlife. Economic losses associated with animal production (abortion "storms," mastitis, metritis, culling, decreased milk production, increased time between lactations, temporary or permanent infertility and barrier to trade in animals) or public health (loss in productivity and costs associated with diagnosis, treatment, research and eradication) can be debilitating to national economies. The annual economic losses due to brucellosis is increasing from 2630 crore India rupee in 2013 due to loss of 2.6 million new female calves per annum to a median loss of 22554 crores Indian rupee as reported by Singh et al., in 2015. It is estimated that an abortion in female animal costs a farmer Rs. 6000/-, which can be easily avoided by successfully implementing control measures. Prevention of abortions will add 2.63 million new female calves per annum valued at Rs. 2,630 crores. Increase in national milk production by 5 per cent at the end of five year will add an annual value of Rs.7,890 crores. The added advantage of controlling the disease would be in the form of reduced infertility, metritis and production losses (Malik et al., 2013). Human brucellosis causing physical incapacity and loss of 3 million man days of labour annually and it is estimated that 5 lakhs new human cases are affected annually in the world. Three species of *Brucella* known to be the principal cause of zoonotic infection in descending order of virulence are *B. melitensis, B. abortus and B. suis.* However *B. canis* has also been occasionally reported to cause infection in humans (Meyer, 1990).

Brucellosis in India

Brucellosis was first recognised In India in 1942 (Radostits et al., 2007). Many reports proved the prevalence of the disease in all the states (Madkour, 2001; Lucero et al., 2008; Fosgate et al., 2011. A recent study on 4,580 animals of 119 dairy farms shows 65.54% (78/119) herd prevalence and 26.50% (1,214/4,580) individual animal prevalence of brucellosis in cows and buffaloes in Haryana and Punjab (Chand and Chhabra, 2013). A study from Uttar Pradesh conducted in 2000 showed an overall prevalence of 7.25% in bovines with 12.77% seropositivity in cattle and 3.55% in buffaloes (Upadhyay et al., 2007). The overall prevalence of bovine brucellosis in Punjab was 11.23% varying from 0% to 24.3% in different villages (Dhand et al., 2005). The PD_ADMAS (2005-2010) reported presence of the disease throughout the country with varying prevalence in different animal species, viz: bovine (22.15%), sheep (8.85%), goat (6.23%), pig (15.35%), yak (16.0%) and mithun (19.0%). This high prevalence of animal brucellosis is responsible for human infections due to close contact with animals. The disease appears to be on the increase in recent times, perhaps due to increased trade and rapid movement of livestock (Renukaradhya et al., 2002) and increase in more susceptible high producing cross bred cattle population.

Risk factors associated with brucellosis

Reasons for endemicity of brucellosis include expanding herds and flocks of livestock and with uncontrolled movements. Trading of animals within and between the states cannot be restricted which facilitates spreading of brucellosis due to movement of animals from one location to another (Renukaradhya et al., 2002). Use of semen (from unscreened bulls) for artificial insemination is considered important factor for dissemination of the disease. Test and slaughter of brucellosis positive animals is also not possible in India because of religious sentiments and test and segregation or culling of infected animals is impractical in private dairy sector due to economic reasons. The disease in cattle seems to be associated primarily with intensive farming practices in large organized animal farms (Smits and Kadri 2005). In rural areas, apart from agriculture, dairy, animal husbandry, free grazing, movement with frequent mixing of flocks of sheep and goats, unrestricted trade and movement of animals, use of local cattle yards and fairs for trading and poor farm hygiene make rural people vulnerable to the spread and transmission of the infection. Also lack of veterinary support services as well as vaccines favours spread of infection.

Vaccination against brucellosis

For over 60 years, the *B. abortus* S19 vaccine has been used in cattle and the *B. melitensis* Rev.1 vaccine has been used in sheep and goats to prevent abortion and infertility caused by natural infection with virulent strains of these *Brucella* species (Moriyon *et al.* 2004). These vaccines, combined with serologic surveillance tests, have been instrumental in the success of the brucellosis eradication programs.

B. abortus S19 vaccine

It is a low virulence, smooth *Brucella* strain which expresses the O side chain (perosmine residue) on its lipopolysaccharides (LPS). This attenuated strain multiplies within the body of the animal for a shorter period of time than the virulent field strain from which it was derived. *B. abortus* S19 lacks the erythritol catabolic gene rendering it sensitive to erythritol (Sangari *et al*., 1998). This strain differs from *B. abortus* biovar-1, the main causative agent of disease in cattle, by its inability to grow in the presence of dye thionine blue, penicillin and erythritol. Reduction in the incidence of disease and resistance to infection are documented in cattle after vaccination with S19. Post challenge reported protection rates, in cattle are 65-75%. Routine vaccination with *B. abortus* S19 is often carried out in calves between 4 to 12

months of age. Vaccination at this age minimizes the presence of persistence antibodies that can interfere with routine serological surveillance tests, which are carried out usually in the animals of breeding age. Vaccination against brucellosis prevents abortions in the herd. Analysis of data collected from organized farms where *B. abortus* S 19 calf hood vaccination program was carried out, revealed that there was gradual decline in abortion rate from 7.96% to 0.93% in cows and 2.53% to 0.68% in buffaloes in last decade. The overall abortion rate in vaccinated cattle was 4.05% and in vaccinated buffalo it was 1.55%. This was the first live vaccine which made virtual eradication of the disease, possible. It is generally agreed that vaccine is essential for the control of bovine brucellosis and in higher prevalence areas, the use of vaccine in terms of protection is more important than its interference in serological tests.

Brucellosis Control Program (B_CP) in India from 2011

- Targets *B. abortus* S19 vaccination for all the female calves of 4-8 months in infected villages with prevalence of greater than 5%.
- The program assures very high and sustained cost benefit ratio to the farmer and dairy industry and helps to establish accredited herds/villages.
- Human infection from bovines is greatly reduced and awareness is enhanced.
- Tagging of vaccinated calves helps to identify the vaccinates and vaccinated animal is valued animal.
- It does not recognize individual infected animal rather it recognizes village as a herd and intends to involve village milk co-operatives in diagnosis and control through vaccination.
- It suggests periodical surveillance using milk ring test for pooled milk samples and ELISA for random serum or herd screening (Area or Survey tests).

Advantages of calf hood vaccination

A. Vaccine produces short lived antibody response upto 6 to 8 months and remain protected till 3rd or more lactations. Vaccinal antibodies disappear by first calving and no repeat or booster vaccination is required and remain negative till re-exposed.
B. Builds up herd immunity in 3-5 years period. Anamnestic response helps animals to act as indicator system.
C. Vaccination costs less than Rs. 25 -30 per calf to be paid by the milk co-operatives/ Govt. Re-infection boosts protection and re-infected animal do not abort and re-infected animal need not be a carrier and it serves as an indicator animal.

Disadvantages of calfhood vaccine:

A. Vaccination of pregnant animals results in abortions and strain 19 vaccination results in the development of an arthropathy in few cases ranging from less than 1 up to 2.5% under field conditions (McDiarmid, 1985).
B. Calf hood vaccine excrete in the semen hence male animals cannot be vaccinated.
C. Calf hood vaccine is infective if proper protective measures are not taken while vaccinating the animals. Accidental injection/ inoculation/conjunctival splashing while adjusting vaccine volumes in syringes / or contact of vaccine on abraded skin results in humans infection.
D. The antibodies are detected in the serological assays used for the diagnosis of brucellosis and are the major problems associated with strain 19 vaccination since they prevent easy differentiation of vaccinated from infected cattle. This is not true because animal is vaccinated at the age of 6-8 months and vaccinal antibody titres persist for 180days (6 months). So after first calving, vaccinal Abs disappears by first calving.

Source of calf hood vaccine (approved vaccine manufactures by DADF, GOI):

1. Bruvax (Indian Immunologicals Limited, Hyderabad) available as live freeze dried S-19 for female cattle and buffalo calves of age 4 to 8 months given 2.0ml subcutaneously.
2. Brucella vaccin**e** (Intervet India Pvt. Ltd., Pune) for cattle and buffalo calves of age 4 to 8 months given 5.0ml subcutaneously.

Role of ICAR-NIVEDI in Brucellosis Control Program:

- ❖ The institute is entrusted to provide ELISA kits to the states and union territories in as and when requested for the program.
- ❖ To train officers of states and union territories if required for effective implementation of the program.
- ❖ To provides logistic support to states and union territories for the verification of suspected and doubtful samples.

Suggested Readings

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11. Clinical Epidemiology of Livestock Diseases

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Clinical epidemiology may be defined as the research discipline concerned with applying epidemiological methods to questions directly relevant to the practice of medicine at the individual or herd/flock level. The sorts of questions asked in the clinical practice of veterinary medicine are listed in Table. 1

Table 1. Clinical issues and Questions in the Practice of Medicine

Issue	Question
Normality/abnormality	What are the limits of normality? What abnormalities are associated with having a disease?
Diagnosis	How accurate are the diagnostic tests or strategies used to find a disease?
Frequency/Occurrence	What is the case definition for a disease; how common are each of the findings? What are the host and spatial and temporal distribution of the disease?
Risk/Prevention	What factors are associated with the likelihood of contracting disease?
Prognosis	What are the consequences of having a disease? What factors are associated with an increased or decreased likelihood of recovering from disease?
Treatment/Control	How effective is a therapeutic strategy and how does it change the future course of a disease? How can the risk and rate of spread of the disease be reduced? How useful are the available tools for diagnosis, treatment, control and prevention?
Cause	What is the etiologic agent? What is its life cycle? What characteristics contribute to its pathogenicity and virulence? What factors determine the susceptibility or resistance of individuals to the disease? What conditions predispose populations to outbreaks?
Source/Transmission	What is the source and reservoir mechanism of the causative agent? What are the periods of communicability? How is the agent spread from infected to susceptible individuals? What is the route of infection?
Cost	What is the impact of a disease in personal and economic terms?

Ref: Fletcher, R.H. et al., Clinical Epidemiology: The Essentials, 2nd ed., Williams & Wilkins, Baltimore, 1988.

The answers to these questions are of immediate relevance to disease diagnosis, risk appraisal, prognosis and treatment.

Study designs may be observational or experimental

A clinical observational study represents a formal approach to which practitioners turn their practical observations into experience. Experimental studies (clinical trials) evaluate the

relative merits of various interventions such as therapeutic, surgical, clinical or preventive approaches to a particular disease syndrome. Clinical epidemiology provides the tools to help practitioners apply their own experiences, the experiences of others fellow vet, and the medical literature to medical decision making. Epidemiologists study disease in its natural habitat, away from the controlled environment of the laboratory. Clinical epidemiology focuses on the sorts of question asked in the practice of medicine.

Application of epidemiology in veterinary practice

Epidemiology has been described as a basic science for clinical medicine (Sackett *et al*., 1991). Epidemiologic studies are often the only way of exploring clinical issues such as

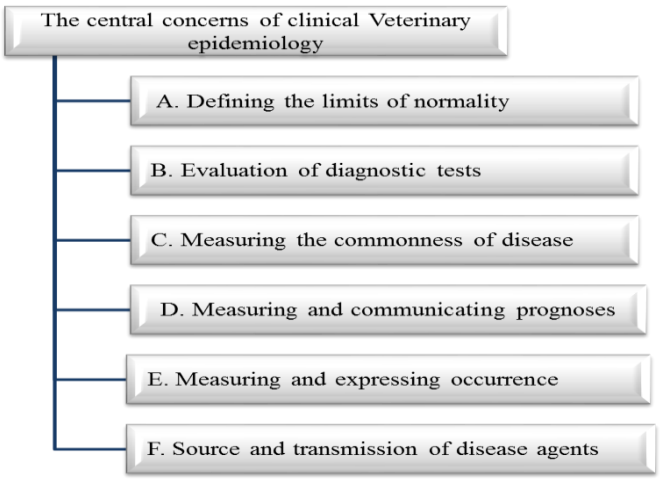
- The accuracy of diagnostic tests,
- Risk factor identification,
- Cause of diseases of multiple or uncertain etiology, and
- Disease prognosis with and without treatment.
- Studying rare conditions or complications of disease that would be difficult to induce experimentally.

One's own patients represent an important source of epidemiologic data. The cumulative clinical experience captured in a patient database can be used to evaluate and improve patient care. Epidemiology also provides the tools for critical evaluation of medical claims. Bias, methodological errors, invalid expectations, and chance can lead to wrong conclusions from clinical studies. As one author put it: "science is the currency of medicine and the standard by which therapeutic claims are judged" (Ramey, 2003). The relationship between epidemiology and clinical medicine has been formalized in the practice of evidence based medicine, the process of systematically finding, appraising, and using contemporaneous research findings as the basis for clinical decisions (NLM, 2004).

Evidence based medicine consists of the following five steps (Sackett et al., 1997)

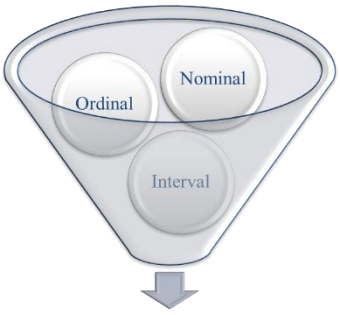
1. At each stage of the case workup, identify one or more clinically important information needs and convert them into answerable questions.
2. Track down, with maximum efficiency, the best evidence with which to answer the above questions.
3. Summarize and critically appraise the evidence found for scientific validity and applicability.
4. Apply the results of this appraisal to patient care
5. Evaluate your performance at answering the questions.

The central concerns of clinical veterinary epidemiology



A. Defining the limits of normality

The process of medical decision making consists of four components: Collection of



Three principal scales used for measuring clinical phenomena

Nominal data can be placed into discrete categories that have no inherent order. Synonyms for nominal data is categorical data. Ordinal data are categorical data with an obvious order that can be ranked, but the intervals are not uniform in size. Data that are ordered and for which the sizes of the intervals are known are called interval or continuous data.

1. **The nominal (classificatory) scale**
   The nominal scale involves the use of numbers (or other symbols) to classify objects. Thus, male and female can be coded 1 and 2
2. **The ordinal (ranking) scale**
   The ordinal scale allows groups to be related to other groups. Most commonly, the relation can be expressed in terms of equal to greater than or less than. Examples are the use of body condition scores for sheep and clinical grading of severity of disease.
3. **The interval scale**

In an interval scale, the distance between the ranked values is known with some accuracy. A good example is body temperature. Two thermal interval scales are commonly used are Celsius and Fahrenheit each containing the same amount of information. The ratio of the intervals are independent of the zero point and are equal to the ratios of the different on the other interval scales. The interval scale therefore includes equivalence, 'greater than' relationships, and ratios of intervals. Because the ratios are independent of the zero point, arithmetic calculations can be performed only on differences between numbers. The interval scale is a relatively 'strong' form of (actual) measurement

B. Evaluation of diagnostic tests

Diagnostic tests play a critical role in decision making process. A difference must be made between diagnostic and screening test scenarios. Diagnostic testing is used to distinguish between animals that have the disease in question and those that have other diseases on the differential list. Diagnostic testing begins with diseased individuals. Screening is used for the presumptive identification of unrecognized disease or defect in apparently healthy population. Screening begins with presumably healthy individuals. The same test, examination, or procedure may be used for either purpose.

Selection of tests based on sensitivity and specificity:

Examples:

1. In the case of mass screening test for Brucella Rapid plate test is preferred. This test is very sensitive. Here, no positive case will be lost, in this case, we don't want to lose positive case even though there are many chances of getting false positive tests. So here look for sensitivity rather than bothering about specificity.
2. In the case where in which we want to detect true negative, we look more specificity. For instance, if we want to cull an animal after testing, we have to go for specificity.

So chances of getting maximum true negative is enhanced and thus culling of any selected animal is prevented.

So decision on appropriate criteria for a screening test depend on consequences of identifying false negative and false positive cases

C. Measuring the commonness of disease

Measurement of the frequency or occurrence of clinical events is fundamental to assessing the risk of contracting of disease, its cause, prognosis, cost of treatment and response to treatment. The frequency of clinical events is usually expressed as a proportion, with cases as the numerator and population at risk as the denominator. These proportions are usually referred to as rates, although the rate is more suitably reserved for those proportions that include a time component. A rate is not the same thing as a ratio. In the case of a rate, the numerator is included in the denominator, while in a ratio, the numerator and denominator are mutually exclusive.

Rate: A rate deals with the frequency (amount) of disease occurrence in a population during a defined time.

$$\text{Rate (R)} = \frac{\text{Number of animals affected during the period}}{\text{Average population of animals exposed to risk during the same period.}}$$

The population rates of affected animals are estimated by calculating the incidence rate (IR) or prevalence rate (PR). They signify two distinct trends of disease occurrence.

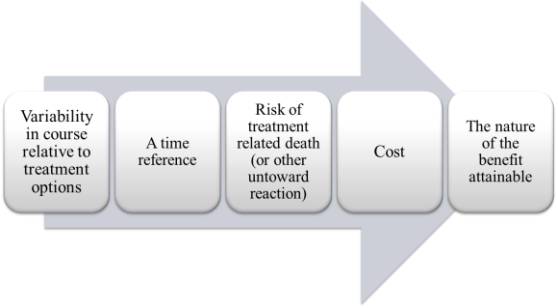
Veterinarians routinely deal with a number of rates (Incidence rate/prevalence rate). Some are vital statistics that can be used to provide indirect evidence of the health status of a population. Others may be classified as morbidity rate, i.e., direct measures of the commonness of disease. Among the rate, the three most commonly used are prevalence, incidence, and attack rate.

Risk assessment and prevention

An understanding of the concept of risk is fundamental to an understanding of such diverse clinical issues as prognosis, treatment, cost of treatment and cause of a disease. Factors that are associated with an increased likelihood of an event occurring (such as disease) are called risk factors. Exposure to risk factors may occur instantaneously (acute) or may be on-going (chronic). Several study designs and analytical techniques can be used to explore the association between presumed risk factors and outcomes.

D. Measuring and communicating prognoses

Prognosis is a prediction of the expected outcome of disease with or without treatment. A prognosis should include



The natural history of a disease describes its evolution without medical intervention. The clinical course of a disease describes its progression once it has come under medical care. The true natural history of unselected cases of a disease, and the course of those that are

recognized, can be quite different. Reports of prognosis from veterinary teaching hospitals and other referral centers may not be representative of cases seen in the typical private practice. Reported cases are often those that had been referred because they were doing badly and not responding to any treatment. It is convenient to summarize the course of disease as a rate (incidence/prevalence rate). All rates used for this purpose are expressions of incidence, e.g., events arising in a cohort of patients over time. Two variables that must be considered in the interpretation of rates are assignment of zero time and interval of follow up. Survival analysis can be used to estimate the average time to event for any time in the course disease. The plotted data are referred to as a survival curve. The most direct way of learning about survival is to assemble a cohort of patients with the condition of interest and periodically assess their status at various time interval throughout the course of their illness.

Maintaining the reliability of a cohort is often difficult in clinical practice because

1. Patients frequently drop out of the study before the end of the follow of period and
2. Patients ordinarily become available for a study over a period of time, leading to variable duration of follow up.

Data on patients (patients drop out of the study) with incomplete follow up are referred to as censored observations. Life table analysis can be used to more efficiently use follow up data, regardless of the time at which an individual enters or leaves a study. With the life table method, the probability of surviving during each time interval is calculated and is used to estimate overall survival through the end of each time interval. The life table approach can be used to describe other outcomes of disease besides death, such as recurrence of tumor, remission duration, graft rejection, or reinfection and to identify prognostic factors for these outcomes.

Points must be considered when interpreting survival curves:

- Since the data include censored observations, the percentage of individuals at each data point may not be equivalent to the actual number of individuals remaining in the study.
- The number of individuals at risk declines as one progresses along the survival curve, which reduces the precision of survival estimates.
- The decreasing slope (or tail) of a survival curve over the follow up period may simply be the effect of a relatively constant survival rate upon a steadily decreasing population at risk.

The use of qualitative terms to express chances of success or failure is inherently unclear. Furthermore, veterinarians frequently do no agree on the prognosis for many common illnesses. There is a clear need for quantitative prognostic information about diseases of domestic animals.

Table 2. Qualitative terms for Clinical Outcomes

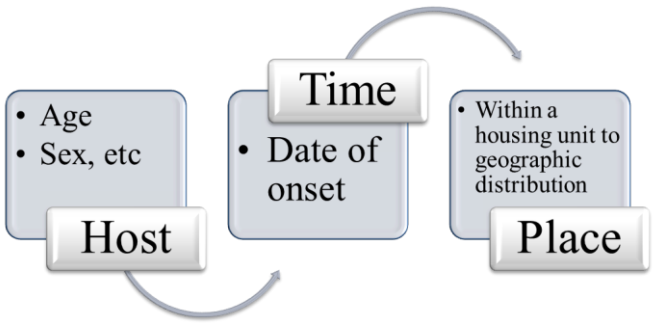
Prognosis	**Probability of Recovery (%)**	**Examples**
Excellent	90-100	Simple indigestion, Mild anaplasmosis
Good	70-89	Intestinal amphistomosis in catttle
Fair	40-69	Septicemic pasteureilosis in cattle
Poor	10-39	Advance stages of pasteureilosis and babesiosis
Grave	0-9	TRP

Crow, S.E., J.Am.Vet. Med. Assoc., 187, 700-703, 1985

E. Measuring and expressing occurrence

Occurrence refers to the frequency distribution of disease over space (spatial or geographic occurrence), time (temporal occurrence), or within a host population. Not only is this information useful to gain a better gratitude of the significance of the disease, but it may suggest the probable cause, source, and mode of transmission. The first step in any disease investigation is identification of the disease (case) and non-disease (non–case). Cases may be defined on the basis of a discrete set of signs and symptoms, performance indicators, or epidemiologic criteria. Epidemiologic criteria, such as the occurrence of the disease, may be added to the case definition.

The occurrence of disease in a population may be reported in three different ways:



An attack rate measures the proportion of the population that develops disease among the total exposed at the beginning of the outbreak. Attack rates are often used to report disease frequency during outbreak investigations. The attack rate is essentially an incidence rate where the time period of interest is the duration of the epidemic.

Attack Rate (AR)

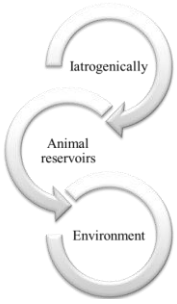
If the course of the disease is short the IR may be called as AR

$$AR = \frac{\text{Number of individuals affected during an outbreak}}{\text{Population at risks at the beginning of outbreak}}$$

AR is useful for identifying risk factors for a specific disease, restricted to outbreak investigation

F. Source and transmission of disease agents

Infections may originate



1. Iatrogenic transmission

Iatrogenic illnesses are those that are induced in a patient by a clinician's actions.

Example:

- Bovine anaplasmosis may also be spread mechanically by infected hypodermic needles, by castrating, spaying and dehorning instruments, and by blood transfusions and embryo transplants.
- In case of babesiosis contaminated needles and surgical instruments can transmit the infection physically. The ease with which infection can be transmitted in this way depends ' largely on the degree of parasitemia occurring with each species. Thus, the chances of physical transmission are slight with *Babesia bovis* and high with *B. equi* and *B. bigemina*.
- Veterinarians and vaccination teams can transmit the classical swine fever virus by contaminated instruments and drugs. The common practice of not changing syringes and needles between farm visits constitutes a major risk when viremic animals are present.

2. Animal reservoirs

Animal reservoirs of disease agents include

1. Carrier animals, animals (including humans) with in apparent infections that are also transmitters or potential transmitters)of the infectious agent, and
2. Intermediate host and vectors.

Amplifying hosts are intermediate hosts that do not suffer from disease, but in which the number of infectious units increases extensively and provides a source for epidemics in humans or domestic animals.

Example:

- Pig acts as an amplifying host for FMD virus.

Animals that have been exposed to an agent may become carriers. Incubatory carriers are capable of serving as a source of infection while incubating the disease. Convalescent carriers continue to shed infectious organisms after the signs and symptoms of disease have disappeared.

Example:

- In cattle infected with FMD virus, carriers may develop during convalescence from the natural disease, or more importantly in vaccinated animals which are exposed to infection. Up to 50% of cattle, sheep and goats may become carriers, but pigs do not. The nasopharynx is the main site for persistence of the FMD virus and erratic low-level excretion may occur for up to 2 years. The virus may also persist in mammary tissue for 3-7 weeks.
- In case of bovine paratuberculosis infected cattle may excrete organisms in the feces for 15-18 months before clinical signs appear.
- In case of bovine leptospirosis even after the clinical recovery, cattle may shed leptospirae in the urine for long periods. All cattle which have recovered from infection may intermittently shed organisms in the urine and act as carriers and may persist for a mean period of 36 days (10 days – 118 days) with the highest excretion rate in the first half of this period.
- In pigs infected with classical swine fever virus is excreted in the urine for some days before clinical illness appears and for 2-3 weeks after clinical recovery. Virus spread via excretions is more important in early stages of an outbreak. Sick pigs excrete virus until they die and obviously even longer if they recover.
- One of the remarkable feature of the Bovine Herpes Virus –1(Infectious bovine rhinotracheitis) is its ability to become latent following a primary infection with field isolate of the virus or vaccination with an attenuated strain. The virus may remain latent indefinitely and recrudescence, reactivation and shedding of the virus may be achieved by the use of large doses of corticosteriods which mimic the effects of stress. Latent infection with virulent BHV-1 virus may occur in the trigeminal ganglion of

calves (previously vaccinated with the modified live vaccine). The virulent virus may spread along the trigeminal peripheral nerve despite the presence of humoral antibodies in vaccinated calves. From trigeminal ganglion the virus spread along the peripheral nerves by intra axonal flow to the nasal mucosa following treatment with corticosteroids /stress.

Diseases are broadly classified as transmissible (communicable) or non-transmissible. Practically speaking, introduction into the herd of an animal afflicted with a non-transmissible disease does not increase the likelihood of disease in others.

3. Horizontal disease transmission

Horizontal disease transmission between contemporaries, or animals of more or less the same generation, may occur directly, indirectly, or via airborne routes. Direct transmission implies direct and essentially immediate transfer of an agent from infected to susceptible hosts. This may occur by direct contact, as through touch, a scratch, lick, bite, or intercourse, artificial insemination. A second mode of direct transmission is through direct projection (droplet spread), where atomized droplets are sprayed onto the conjunctiva or mucous membranes of the eye, nose, or mouth during coughing or sneezing.

Examples:

- In case of classical swine fever the source of virus is always an infected pig or its products and the infection is usually acquired by ingestion but inhalation is also a possible portal of entry. Direct animal to animal contact is the most important method of spread. Infected pigs shed a large amount of the virus in all normal secretions - nasal, salivary, urinary, fecal and is important in transmission when there are clinical signs of the disease. In endemic areas, transmission to new farms can occur in feeder pigs purchased for finishing, or indirectly by flies and mosquitoes, or on bedding feed, boots, automobile tires or transport vehicles.
- In case of infectious bovine rhinotracheitis in cattle the main sources of infection are the nasal exudates and coughed up droplets, genital secretions, semen, and fetal fluids and tissues.
- In case of peste des petits ruminants in goat close contact with an infected animal or contaminated fomites is required for the disease to spread. Large amounts of the virus are present in all body excretions and secretions, especially in diarrheic feces. Infection is mainly by inhalation but could also occur through the conjunctiva and oral mucosa.

Airborne transmission involves the dissemination of microbial aerosols in the form of droplet nuclei or dust. Droplet nuclei are the small residues that result from evaporation of fluid from droplets emitted by an infected host/animal. They may also be created by atomizing devices, accidentally in microbiology laboratories, abattoirs, rendering plants, or necropsy rooms. Droplet nuclei usually remain suspended in the air for long periods. Dust consists of the small particles of widely varying size that arise from soil (as fungus spores separated from dry soil by wind or mechanical agitation), clothes, bedding, or contaminated floors and contaminated utensils.

Example:

- In case of FMD virus spread between cattle is more likely to be by airborne means. The virus can persist in aerosol form for long periods in temperate or subtropical climates but not in hot and dry climates. The speed and direction of the wind are important factors in determining the rate of airborne spread.

Indirect transmission may be vehicle-borne or vector-borne. Vehicle-borne transmission occurs through exposure to contaminated inanimate objects (fomites), such as bedding,

surgical instruments, soil, water, food, milk, and biological products (including blood, serum, plasma, discharge, tissues, or organs). The agent may or may not have multiplied or developed in or on the vehicle before being transmitted.
Examples:

- In case of *Bacillus anthracis* infection gains entrance to the body by ingestion, inhalation, or through the skin. While the exact mode of infection is often in doubt, it is generally considered that most animals are infected by the ingestion of contaminated food or water. The increased incidence of the disease on sparse pasture is probably due both to the ingestion of contaminated soil and to injury to the oral mucosa facilitating invasion by the organism. Inhalation infection is thought to be of minor importance in animals, although the possibility of infection through contaminated dust must always be considered.
- In case of disease associated with brucella species the disease is transmitted by ingestion, penetration of the intact skin and conjunctiva, and contamination of the udder during milking. The organism does not multiply in the environment but merely persists, and the viability of the organism outside the host is influenced by the existing environmental conditions. Grazing on infected pasture, or consuming other feedstuffs and water supplies contaminated by discharges and fetal membranes from infected cows, and contact with aborted fetuses and infected newborn calves are the most common methods of spread. Intraherd spread occurs by both vertical and horizontal transmission. Horizontal transmission is usually by direct contamination and, although the possibility of introduction of infection by flies, dogs, rats, ticks, infected boots, fodder, and other inanimate objects exists.
- In case of clostridial infection in sheep is almost always a wound infection. Infection of skin wounds at shearing and docking and of the navel at birth may cause the development of local lesions. Infections of the vulva and vagina of the ewe at lambing may cause serious outbreaks and the disease has occurred in groups of young ewes and rams up to a year old, usually as a result of infection of skin wounds caused by fighting.
- In case of brucella infection the most likely and effective means of cattle-to-dog transfer is exposure to aborted fetuses or infected placental membranes, because dogs commonly ingest the products of parturition.

Vector-borne transmission is generally understood to mean transmission by invertebrate vectors, such as files, mosquitoes, ticks. It may be mechanical or biological. Mechanical transmission results from simple mechanical carriage of the disease agent between hosts by crawling or flying arthropods. It does not require multiplication or development of the disease agent in the vector.
Example:

- In case of trypanosomosis several hematophagous flies can transmit *Trypanosoma evansi* mechanically, the most important is the horse fly (*Tabanus* Spp.), followed by the stable fly (*Stomoxys* spp).

Biological transmission requires a period of multiplication, cyclic development, or both before the vector can transmit the infective form of the agent. The disease agent may be transmitted vertically (transovarially) between generations of the vector or transstadially from one stage to another within a single generation.
Example:

- In case of bovine anaplasmosis a variety of arthropods may cat as vectors but significant natural vectors are ticks in the family *Ixodidae* and flies in the family *Tabanidae*. Of the ticks, the one host Boophilus spp., are of major importance in

tropical and subtropical regions. The organism undergoes a complex developmental cycle in the gut cells of ticks and the final infective stage is present in the salivary gland. Transstadial transmission of the organism occurs in ticks.

- Bovine ephemeral fever is transmitted by mosquitoes *Aedes* spp., *Culex annulirostris, Anopheles bancroftii* and *A. annulipes,* and the biting midge *Culicoides brevitarsis*. This mosquito can transmit infection within a week of feeding on an infected animal.
- In case of blue tongue in sheep is not contagious and is transmitted biologically by certain species of *Culicoides.* There are over 1000 species of *Culicoides* worldwide but only a limited number have been associated with Bluetongue virus. They feed nocturnally on animals in open pens and fields and the optimal temperatures for activity lie between 13°C and 35°C. Virus is ingested blood infects cells of the midgut and by a receptor mediated process, replicates and subsequently released to the salivary gland. The cycle of transmission in the insect takes 10 – 15 days and the vector is infected and infective for life. Bluetongue virus is maintained in nature by alternating cycles of infection between the midge and ruminant species.
- Sheeppox and goatpox are highly contagious and, although in most cases spread appears to occur by contact with infected animals and contaminated articles, spread by inhalation also occurs.

Endemic stability is defined as the state where the relationship between host, agent, vector and environment is such that clinical disease occurs rarely or not at all. Endemic stability (herd immunity) in bovine babesiosis occurs when the rate of transmission (inoculation rate) of Babesia spp. by the tick vector is sufficient to immunize a majority of susceptible calves before the loss of calfhood resistance. In tropical areas with a high vector population, natural exposure usually occurs at an early age and cattle are therefore immune to subsequent challenges as adults. If at least 75 % of calves are exposed to *B. bovis* infection by 6 to 9 months of age the disease incidence will be very low and a state of natural endemic stability would exist.

4. Vertical transmission

Disease transmission may also occur vertically from animals of one generation to another generation. Vertical transmission may be transovarial, e.g., between generations of invertebrate vectors via the egg, in utero, or transplacental (from parent to offspring within the uterus) or colostral (from parent to offspring at parturition via colostrum or milk).

Examples:

- *Toxocara vitulorum* larvae are present in greatest number in the colostrum 2-5 days calving and few are present after day 9. Mature worms are present in the intestine of the calf by 10 days of age and eggs are passed by 3 weeks.
- In case of *Mycobacterium avium* subspecies paratuberculosis (Map) the most common route of transmission is through nursing from an infected dam (via contaminated teats or direct shedding of the organism into the colostrum/milk). Infected cows and other species excrete Map directly into the milk during at least the late disseminated stage of the infection. Up to 45% of clinically affected cows may excrete the organism in milk. The organism can be found in the colostrum of subclinical cases; the organism was found in 36% of colostrum samples from heavy shedders and 9% of samples from light shedders, nearly three times as often as it is found in mill. Thus colostrum of infected cows, if fed to calves, could serve as a potential source of infection.
- In case of bovine virus diarrhea virus (BVDV) disease occurs only in Persistently-infected (PI) animals as a result of a congenital infection with a non-cytopathic strain of the virus acquired in early fetal life. These animals remain specifically immunotolerant to the homologous strain of the BVDV throughout postnatal life, and

fatal mucosal disease is precipitated by a superinfection with a cytopathic strain of the virus occurring usually at 6-24 months of age or older. Young cattle which are persistently infected with a non-cytopathic strain of the virus are the major source of infection in a herd.

- In case of diseases associated with brucella species congenital infection may occur in calves born from infected dams but its frequency is low. The infection occurs in utero and may remain latent in the calf during its early life; the animal may remain serologically negative until its first parturition, when it then begins to shed the organism, Calves born from reactor dams are serologically positive for up to 4-6 months because of colostral antibodies and later become serologically negative even though a latent infection may exist in a small proportion of these calves. The frequency of latent infections is unknown, but may range from 2.5-9%.

Communicability may be defined as the ease with which a disease agent is spread within a population. One way of expressing communicability is the intrinsic (or basic) reproductive number, which represents the average number of secondary infections generated by one primary case in a susceptible population and can be used to estimate the level of immunization or other risk reduction strategy required to control an epidemic. Communicability is affected by agent, host, and the minimal infective dose. Host factors may appear as heterogeneity in susceptibility to disease due to innate or immune factors. Environmental factors include particle diameter and the microclimate in which the infectious agent resides.

Key messages

- Clinical Veterinary epidemiology is the application of epidemiological principles and methods to the practice of Veterinary medicine.
- With rising health-care costs, clinical practice has become a common subject of epidemiological research.
- Evidence-based guidelines have improved clinical outcomes.

Suggested Readings

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12. Importance of Surveillance and Monitoring: Role of Field Veterinarians

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India has huge livestock population of 512 million, including 299.9 million bovines; 65.6 million sheep; 135.2 million goats; 10.3 million pigs; 1.15 million horses and mules; 0.4 million camels; 729.2 million poultry and others (19th National Livestock Census, 2012). Livestock health-care is provided by 52,757 veterinary service hospitals / dispensaries (27,050 rural veterinary units; 22,745 veterinary dispensaries and 11,101 veterinary hospitals and polyclinics), primarily run by state governments provide variety of veterinary and breeding services. These are also differently supported by large number of veterinary colleges, five Regional Disease Diagnostic Laboratories (RDDLs) of GOI and many ICAR institutions. The national veterinary manpower is over 100,000 veterinarians and Para-veterinary staff. Over 75 percent of these staff is committed to treatment, vaccination, breeding and extension activities.

Large number of endemic infectious (bacterial, viral and protozoan) and parasitic diseases inflict considerable economic losses through morbidity, mortality, decreased production, reduced fertility, inefficient feed utilization resulting in inadequate weight gain and impaired draught power. Further, some of these diseases are zoonotic and have significant impact on public health especially to womenfolk, who traditionally work with animals. Unpredictable agrarian conditions influenced by vagaries of weather, draught, floods, livestock migratory habits, poor animal nutrition, zoo-sanitary and inadequate preventive healthcare practices and lack of national disease control policies have perpetually contributed to high incidence and sustained prevalence of livestock and poultry diseases, often round the year. Also, regional animal husbandry, migration and management practices under local diverse agro-climatic conditions play critical role in sustenance of location-specific livestock diseases in the country. In the Indian context, most livestock diseases are highly location-specific although Foot and Mouth Disease is truly 'Transboundary' and outbreaks occur round the year in most parts of the country - though considerably reduced level due to national vaccination campaign sponsored by Govt. of India. In the light of the preceding, this paper attempts to explore key role played by Field Veterinarians in livestock disease monitoring and surveillance that leads to improved national livestock health management through disease prevention and control action plans.

Concepts of Disease Monitoring and Surveillance of livestock diseases

Surveillance is a population based disease intelligence activity that aims for early detection of subtle or frank cases, often new disease incursions, by collection, confirmation, analysis, and interpretation of epidemiological data. In recent times, the usage of 'disease surveillance' has found greater global acceptance to include 'disease monitoring' which was hitherto conventionally restricted to deal with analysis of endemic disease situations, their epidemiological estimates and short and long term trends, with or without preventive interventions. The goal of surveillance is to evolve long term specific actions in the prevention and control of diseases.

The success of an effective disease surveillance system (including disease monitoring) largely depends on the simplicity of immediate disease outbreak reporting, disease

confirmation, estimation of population-risk parameters and epidemiological investigations to establish host-pathogen-environmental impacts. Disease surveillance is a continuous sustained national effort to keep vigil on endemic diseases of economic and zoonotic importance and in particular, 'Transboundary diseases' across the international borders.

Well-designed disease surveillance system provides to ascertain:

- population disease magnitude (incidence and prevalence),
- long and short term disease trends,
- epidemiological interfaces that need attention,
- evolve disease prevention/control strategies,
- assessment/impact of on-going disease prevention/control situations,
- warn the vet administrators to initiate control activities.

Constraints in disease surveillance activity

The livestock sector is always troubled with high magnitude of endemic diseases despite the country having reasonably accessible 52,757 network of veterinary dispensaries/hospitals to meet healthcare of 512 million heads of livestock in 6,36,000 villages. Though reasonable, the number of veterinary institutions and professionals are by and large inadequate to the challenges of high endemic diseases to evolve an organized dedicated, result oriented, effective and efficient disease monitoring and surveillance system in the country.

Diseases being dynamic, the past system of disease recording, reporting, storage, retrieval and transmission of analyzed information to the user agencies, which was primitive, lethargic, nonresponsive and counterproductive, has gone through a sea of change by the pioneering research initiated and lead by PD_ADMAS/NIVEDI in the past two decades to the present National Animal Disease Reporting System (NADRS) - now implemented by Govt. of India. Presently, lack of timely and reliable laboratory validated disease confirmation continues to be a perpetual problem, though inputs form RDDLs is visible in recent times. The following are some of the major constraints.

The existing disease reporting system is optimized at all levels

- Clinical symptoms based disease reports, often lack lab confirmation
- There is severe dearth of lab infrastructure, quality and availability of diagnostics and trained manpower for disease confirmation.
- Under reporting and not-reporting are rampant (order of the day!!).
- Disease data are more subjective, and lack detailed investigation
- Disease data compilation is incomplete and inadequate, as data from NGO's, private practitioners, universities sources are not included.
- There is no national cohesiveness in handling disease data as each state acts as a 'compartment' without any interaction with others
- Lack of sustainable national surveys on all endemic diseases, their trends and impact of preventive vaccination
- Veterinary extension education for farmer participation is inadequate

Ideal national disease surveillance – rationale

The active surveillance of diseases is essentially early recognition of the disease outbreak, its laboratory validation, tracking of its source and movement coupled with identification of precipitating factors which will provide clues for the critical control measures to the logical end of the outbreak. This leads to reduce establishment of a temporary or permanent endemic disease foci. The basic rationale for sustainable disease surveillance is through organized

nation-wide continuous observation on disease behavior in livestock population. Surveillance evaluation reports generated through retrospective and prospective disease situations are useful for:

- geographic and seasonal estimates of diseases,
- establish indices for projection of future disease patterns and trends,
- develop time and location specific epidemiological profiles,
- forewarn the endemic, new or emerging diseases,
- evolve strategies for national disease control and eradication,
- design economically feasible livestock health delivery system
- promote production and exports,
- conduct risk analysis for import regulation of animals / products.

Role of Field Veterinarians in Disease Monitoring and Surveillance

Indeed, Field Veterinarians are the grass-root level 'bare-foot Epidemiologists' of the country, a great work-force to reckon with. In their day-to-day veterinary activity, they unassumingly create (often unconfirmed) sustainable raw disease data-base on seasonal disease incidence (also, any unseasonal), maintenance and spread within their dispensary / hospital jurisdiction. In turn, these factors collectively form core and critical aspects required to develop effective disease monitoring and surveillance at taluk/mandal/, district, region, state and national levels. It is utmost important that Para-vets and veterinarians at field level are made fully aware that a disease outbreak is absolutely a location and time specific event and occurs for sole purpose of pathogen survival in the animal population. Disease outbreak is an intricate interaction between pathogen and host supported by congenial environment that helps pathogen perpetuation and spread in the population. Below are some important considerations for Field Veterinarians:

1. Not reporting and under-reporting of disease outbreaks is a global phenomenon, predominantly for trade and economic considerations. India is not an exception to this and often disease reporting attracts wrath of local vet administration – discouraged. There is an urgent need to change the mindset of Para-vets and other veterinary functionaries at various administrative levels that disease reporting is their first and foremost obligation and without any delay. Any slackening and not timely reporting should be viewed seriously officially and any lapse is followed by enquiry and warnings.

2. On priority, Para-vets at rural animal health units must be encouraged to personally go over to taluk vet institution to report disease outbreaks to save on time and to get directives to effectively deal with the disease. Alternately, disease outbreak information should be conveyed through mobile/telephone, where facilities are available. These incidental expenses incurred are reimbursed. Animal owners help can also be sought where possible and their participation goes a long way in timely health care delivery service.

3. Field Veterinarians need basic training in handling of disease outbreak data including estimation of population at risk, collection of clinical samples for lab investigations, segregation/quarantine and movement restriction and vaccination/control approaches need to be imparted to supportive field functionaries. A Standard Operating Procedure (SOP) for disease monitoring and surveillance should be made available at all peripheral vet institutions.

PD_ADMAS (NIVEDI) experience with diseases reported by Field Veterinarians:

- A treasure of national livestock disease information.

Since inception in 1987, this institution was seriously questioned in every veterinary forum on the credibility and validity of disease outbreaks reported by field veterinarians. One of the

serious doubts was that outbreaks were not laboratory confirmed and lacked epidemiological core details of morbidity, mortality, source, spread of infection and population at risk.

The then prevailing field veterinary services situations (probably, even now) in the country, it was impossible to expect ideal epidemiological investigations of disease outbreaks. Considering these ground realities, PD_ADMAS was pragmatic to vehemently defend and pursue that the disease outbreaks were indeed time-and-location specific events and population parameters, though important could not be integrated in the Indian context of huge livestock population, varied, zoo-sanitary, migration, husbandry-management practices and limited resources. It also strongly held the view outbreaks occur for absolute valid reasons that support survival, spread and maintenance of the pathogen in the livestock population, at specified temporal and spatial equations.

With this conceptual approach, PD_ADMAS created a 10-year national computer databases on livestock populations, infectious diseases, species affected, morbidity, mortality, and temporal and special factors, migration, meteorological, soil profiles, veterinary institutions and village registry. This was huge effort and as anticipated yielded most valuable short and long term disease incidence and prevalence at district-state-national levels. Several critical aspects of diseases were frankly elucidated to initiate action plans for prevention and control. This has led to better understanding of location-and-time specific disease precipitating factors.

A well-document in-depth analysis and interpretation of such 10-year of disease outbreak data recorded by Field Veterinarians of Andhra Pradesh state is presented in my Consultancy Report**:** FAO Pro-Poor Livestock Initiatives on " Control Strategy and Action Plan for Animal Diseases of Economic Importance in Andhra Pradesh - 2008". *(Food and Agriculture Organization (FAO), Rome Web site: www.fao.org/ag/againfo/projects/en/pplpi/home.html).* The outcome of this analysis is indeed a tribute to thousands of Field Veterinarians who unassumingly and painstakingly routinely carryout disease reporting, which has immense value in national disease monitoring and surveillance and initiation of disease prevention and control.

13. Introduction to Open Source Epidemiological Software and its Applications

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Open source software (OSS) refers to software that is developed, tested, or improved through public collaboration and distributed with the idea that it must be shared with others, ensuring an open future collaboration. The collaborative experience of many developers, especially those in the academic environment, in developing various versions of the UNIX operating system, Richard Stallman's idea of Free Software Foundation, and the desire of users to freely choose among a number of products - all of these led to the Open Source movement and the approach to developing and distributing programs as open source software.

Various Epidemiological software's are available and have different applications and uses.
ActivEpi - A collection of tools for learning epidemiology.
Birtha - Vitalnet software for analyzing birth data.
Cancer-Rates.Info - Cancer Incidence and Mortality Data.
CDC Wonder - Public health information system. Free.
CI*Rank - Providing Confidence Intervals for Ranks
Cytel - Epidemiological computing software.
Disease Surveillance On-Line - Disease statistics in Canada.
DoEpi - Teach epidemiology and Epi Info. Free.
EpiCalc 2000 - Statistical calculator for pre-tabulated data. Free.
EpiData - Data entry and data documentation. Free.
EpiGram - Create tracing and social network diagrams.
Epigram - Vitalnet software for analyzing mortality data.
Epi Info - Make questionnaire; enter data; analyze data. Free.
Epi Info PD - Public-domain version of EpiInfo.
EpiMeta - Performs meta-analysis. Free.
Epimonitor.net - Publishes epidemiology newsletter and resource guide.
Episurv - A generator of epidemiologic applications.
Epitools - R package for epidemiologic computing and graphics.
EpiWorld - Discussion Group listserv.
ePrognosis - Estimating Prognosis for Elders
FP Advisor - Helps foodborne disease investigation.
Georgia OASIS - Accesses Georgia health data.
GeoViz - Geographic Data Visualization.
i2b2 - Informatics for Integrating Biology and the Bedside.
iEpidemiology - iPhone app for analysis of epidemiologic study data. Free.
IHME - Institute for Health Metrics
Life Table Analysis System - Occupational cohort mortality studies.
MapWindow - MapWindow GIS Open Source Software.
MassCHIP - Massachusetts Community Health Information Profile.
MassHEIS - Massachusetts Health and Environment Information Study.
MedTrend - Vitalnet software for analyzing hospital discharge data.
Missouri MICA - Missouri Information for Community Assessment.
MIX - Meta-analysis learning; teaching; and exploration.
NetEpi - Open source tools for epidemiology and public health.

Oncogram - Vitalnet software for analyzing cancer incidence data.
Ohio DW - Ohio DOH Data Warehouse.
OpenClinica - Open source clinical trials software.
OpenEpi - An online epidemiologic and statistical calculator.
OpenSource GIS - Index of open source GIS projects.
PEPI - Computer Programs For Epidemiologic Analysis.
PopTrend - Vitalnet software for analyzing population data.
PregData - Vitalnet software for analyzing pregnancy data.
Public Health Associate - Builds differential diagnosis lists of infectious diseases.
Qgis - a free and open source Geographical Information system
Real-Statistics.com - Real Statistics Using Excel
SampleLQ - Sampling plan calculator for lot quality assurance sampling surveys.
SampleRate - Sample size calculator.
SampleXS - Sample size calculator for cross-sectional surveys.
Sampsize - A utility to compute sample size for surveys.
SaTScan - A free software that analyzes spatial temporal and space-time data.
Sentiweb - Interactive queries of communicable diseases in France.
SigmaD - Assists in standardising epidemiological measurements.
SDA - Survey documentation and analysis - UC Berkeley.
SPMA - Standardized Procedures for Mortality Analysis - Belgium.
StatPages.org - Web Pages that Perform Statistical Calculations.
Survey Analysis in R - Thomas Lumley at UW
Texas Cancer Data Center - Online cancer information.
Utah Hospital Discharge Query System - 1 - Descriptive statistics section.
Utah Hospital Discharge Query System - 2 - Hospitalization rates section.
Utah Office of Health Care Statistics - Utah health care information.
Vitalnet - Software and services for analyzing health-related data sets.
WEAT - Web Enabled Analysis Tool - BRFSS.

Epi Info

Epi Info is a free program available from the Centers for Disease Control and Prevention, Atlanta, GA (www.cdc.gov/epiinfo). The program allows the creation of data entry systems and the analysis of data. Version 7 of Epi Info can be copied onto a Windows-based computer without the need to run an install and can be run from a thumb drive. Version 7 is a complete rewrite of the previous Version 3 of the program with a number of improvements.
Epi Info is a free software package developed by the United States of America Centers for Disease Control which allows users to:

- check the survey data for outliers and inconsistent data
- conduct a descriptive analysis of survey data
- easily generate output files from the analysis.

OpenEpi 3.01

OpenEpi (www.OpenEpi) is a free, web-based, open source, operating system-independent series of programs for use in epidemiology, biostatistics, public health, and medicine, providing a number of epidemiologic and statistical tools for summary data. OpenEpi was developed in JavaScript and hypertext markup language (HTML) and can be run in browsers supporting these languages, such as Microsoft Internet Explorer, Mozilla Firefox, Safari, Chrome, and Opera, and on a number of operating systems, such as Microsoft Windows, Macintosh, Linux, and Android tables and smartphones. The program can be run from the

OpenEpi website or downloaded and run without a web connection. The source code and documentation is downloadable and freely available.
Updates in Version 3.01 include

- In addition to English, French, Italian, and Spanish, OpenEpi can now be run in Portuguese
- The program has been modified so it can be used with greater ease on smartphones and tablets
- Added epidemiologic and statistical tests
- The output from a module is all stored in one HTML file during calculations. For example, if several sample size calculations are performed for various scenarios, all of the output is placed in one output HTML file.

OpenEpi provides statistics for counts and measurements in descriptive and analytic studies, stratified analysis with exact confidence limits, matched pair and person-time analysis, sample size and power calculations, random numbers, sensitivity, specificity and other evaluation statistics, R x C tables, chi-square for dose-response, and links to other useful sites. Test results are provided for each module so that you can judge reliability, although it is always a good idea to check important results with software from more than one source. Links to hundreds of Internet calculators are provided.

The programs have an open source license and can be downloaded, distributed, or translated. Some of the components from other sources have licensing statements in the source code files. Licenses referred to are available in full text at OpenSource.org/licenses. OpenEpi development was supported in part by a grant from the Bill and Melinda Gates Foundation to Emory University, Rollins School of Public Health.

WinPepi 11.32

WinPepi (www.brixtonhealth.com) is a free 'Swiss army knife' set of seven Windows-based programs (comprising 124 modules) that provide most of the statistical procedures commonly used in the planning and analysis of epidemiological studies (including meta-analyses), in teaching programs on statistics in epidemiology, and in clinical epidemiological practice. It also includes many procedures that are less commonly used or not very easily found, such as the capture-recapture method, appraisal of the effects of misclassification, multiple significance tests, and unmeasured confounders, the assessment of inter-rater and intra-rater reliability, the use of Bayes factors to appraise whether associations are worthy of note, and estimation of the probability that an effect will be replicated in other studies.
A 'portal' permits easy identification of, and access to, the required modules. WinPepi is user-friendly, provided that users focus on the specific modules and results that interest them, and disregard the many others. The programs are accompanied by extensive manuals that discuss the uses, limitations and applicability of the procedures, and furnish formulae and references. WinPepi does not provide data-management facilities; it usually requires previously-summarized data, entered at the keyboard or pasted from a text file or spreadsheet. The programs can be run from a portable device such as a USB flash drive. The latest version is 11.32.

EpiTools

This EpiTools epidemiological calculators software has been developed by AusVet Animal Health Services, with funding from the Australian Biosecurity Cooperative Research Centre.

The site is intended for use by CRC members and other epidemiologists and researchers involved in estimating disease prevalence or demonstrating freedom from disease through structured surveys, or in other epidemiological applications.

Surveillance utilities

- 1-Stage representative freedom surveys New menu
- 2-Stage representative freedom surveys New menu
- Risk-based freedom surveys
- Random Sampling from a population
- Estimating true prevalence
- Pooled prevalence calculator
- Survey Toolbox for livestock diseases and freedom in finite populations
- HerdPlus module for herd-sensitivity and freedom in finite populations

Epidemiological studies

- Sample size calculations
- Summarise categorical or continuous data
- Statistical significance testing
- Probability distributions
- Bioequivalence analysis

Diagnostic tests

- Application of diagnostic tests

There are many software's available for epidemiological analysis, which can be used for specific applications and purpose. Hence, one has to select the OSS based on his need and applications.

Suggested Readings

1. Sergeant, ESG, 2015. Epitools epidemiological calculators. AusVet Animal Health Services and Australian Biosecurity Cooperative Research Centre for Emerging Infectious Disease.
2. http://www.who.int/chp/steps/resources/EpiInfo/en/ (Accessed on 7.11.15)
3. http://www.ehdp.com/links/episw.htm (Accessed on 7.11.15)

14. Bacterial Mapping of Mastitis

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Dairy industry is of crucial importance to India. The country is the world's largest milk producer, accounting for more than 13% of world's total milk production and for over half the total milk output of Asia. Across India, more than half of all milk is produced from buffaloes. Over 90 % of milch animals (45 % indigenous cattle, 55 % buffaloes, and 10 % crossbred cows) are reared in unorganized sector, wherein, the herd sizes are small and animals are reared in semi-intensive or extensive systems. Cross-bred cattle numbers are increasing but they still account for less than 14% of the total cattle population. Though India is the highest milk producer in the world but the per capita availability of milk still remains half of the world average, demanding strategic intervention. One of the reasons for low productivity is Bovine mastitis.

Mastitis, the inflammation of the mammary gland and udder tissues can be caused by many types of injury including infectious agents and their toxins, physical trauma or chemical irritants. It is the outcome of interaction of various factors associated with the host, pathogen(s) and the environment. Problem of mastitis remains insurmountable to the dairy farmers and poses several challenges to the modern veterinary practitioners. Ten years back FMD was most challenging disease in high yielding dairy animals in India (Varshney and Mukherjee, 2002). But today undoubtedly mastitis is the most challenging disease of dairy animals. Mastitis causes heavy economic losses to the dairy industry. Many factors make up the costs of mastitis. Those most common are decreased milk production, drugs, veterinary services, diagnostics, discarded milk, labour, decreased product quality, increased risk of new cases of the same disease or of other diseases, increased risk of culling, and materials costs required for prevention. It is proved by the reports that the annual economic losses due to bovine mastitis were increased about 135 folds in India. The first report on mastitis quotes that losses was about Rs.52.9 crores annually (Dandha and Sethi, 1962). These losses increased to Rs.6053.21 crores annually in the year 2001 (Dua, 2001). The latest report attributes a total loss of Rs. 7165.51 crores to the Indian economy due to mastitis (Bansal and Gupta, 2009).

Etiological agent of bovine mastitis

Mastitis can be caused by over 250 different microorganisms such as Gram-positive cocci, Gram-negative bacilli (Coliforms especially *E. coli*, *Enterobacter*, *Klebsiella* spp.), *Corynebacterium*, *Mycoplasma*, *Pasteurella*, *Leptospira*, *Yersinia*, *Mycobacteria*, *Pseudomonas*, *Serratia* etc. and other miscellaneous organisms, which include *Nocardia*, *Prototheca* and Yeast (Kuang *et al*., 2009). Of these, the most common mastitis causing pathogens are *Staphylococcus aureus, Streptococcus agalactiae,* coliform and environmental *Streptococcus* spp. (Lee *et al*., 2008). The microorganisms causing mastitis can be grouped into three categories: Contagious (*S. aureus, S. agalactiae, C. bovis, Mycoplasma* spp.), Environmental (*E. coli, K. pneumonia, K. oxytoca, Serratia* spp., *Citrobacter* spp., *S. uberis, S. bovis* and *S. dysgalactiae*) and Others viz., Coagulase negative staphylococci (CNS), *Pseudomona aeruginosa, Nocardia asteroids,* etc (Sudhan and Sharma, 2010).

Mastitis due to Staphylococci

S. aureus is recognised as the most common and frequently isolated aetiological agent of bovine mastitis worldwide (Olde Riekerink *et al.,* 2006). *S. aureus* is ubiquitous and can colonize the skin as well as the udder. It is capable of causing peracute, acute, subacute, chronic, gangrenous and subclinical types of mastitis. The acute form of the disease usually occurs shortly after parturition and tends to produce gangrene of the affected quarters with high mortality. CNS is the part of normal skin flora and has been previously regarded only as minor pathogens. However, the proportion of CNS mastitis has markedly increased over the last decades and is now regarded as emerging mastitis pathogens (Pyorala and Taponen, 2009). However, the significance of CNS needs to be reconsidered as in many countries they have become the most common mastitis causing agents (Tenhagen *et al*., 2006). *Staphylococcus epidermidis, Staphylococcus simulans, Staphylococcus saprophyticus, Staphylococcus hyicus, Staphylococcus warneri, Staphylococcus chromogenes, Staphylococcus sciuri* and *Staphylococcus xylosus* are the commonly encountered species of CNS in bovine mastitis (Tiwari *et al*., 2013).

Mastitis due to Streptococci

Streptococcus agalactiae, S. dysgalatiae and *S. uberis* have been reported as the three most common etiological agents of mastitis (Khan *et al*., 2003). *Streptococcus agalactiae* has been widely reported as an important pathogen causing contagious mastitis. Other Streptococcal species such as *S. bovis, S. acidominimus, S. alactolyticus, S. canis, S. equi*, *S. equinus* and *S. parauberi*s have been implicated in bovine mastitis, although they are relatively infrequent (Khan *et. al.,* 2003). All of these organisms (other than *S. agalactiae*) may be contracted by the cow from the environment and not just from another cow, thus they are called environmental streptococci. These bacteria are found in bedding, soil, walkways or any surface the cow is in contact with. Environmental streptococcal mastitis may be apparent (clinical) or inapparent (subclinical).

Mastitis due to Escherichia coli and Mycoplasmas

Mastitis caused by *E. coli* is common in high-producing cows with low milk somatic cell count. Environmental mastitis caused by *E. coli* has increased in many countries and herds (Peeler *et al*., 2003). The *Mycoplasma* spp. that have been associated with mastitis are considered contagious in nature, transmitted at time of milking from a reservoir, the infected udder; via fomites, hands of a milker, milking unit liners, or udder wash cloths. With respect to *Mycoplasma* mastitis, *M. bovis* is the predominant causative agent and *M. californicum* and *M. bovigenitalium* appear to the next most common. Other species that have been noted as causes of *Mycoplasma* mastitis include *M. arginini, M. bovirhinis, M. canadense* and *M. dispar*.

A large number of other bacteria such as *Brucella melitensis, Corynebacterium bovis, Enterobacter aerogenes, Klebsiella oxytoca, Klebsiella pneumoniae, Pasteurella* spp., *Proteus* spp., *Pseudomonas aeruginosa, Trueperella pyogenes* (previously *Arcanobacterium pyogenes*), etc have been reported to cause mastitis. Mastitis caused by *Pseudomonas aeruginosa* rarely develops in cattle, and occurs only as sporadic form following intramammary infusions of contaminated material. Mastitis caused by *Mannheimia haemolytica* and *Pasteurella* spp. are more common in sheep, but is rarely reported in cattle and is usually sporadic. Other mastitis that evolves sporadically in a herd, affecting one or two cows, can be caused by *Nocardia spp*. and *Serratia* spp. *Bacillus cereus* and *Bacillus subtilis* are saprophytic organisms, occasionally causing acute hemorrhagic mastitis in cattle. Mastitis caused by *Listeria monocytogenes* is important because of the zoonotic risk due to

the consumption of contaminated dairy products. Also, mastitis can be rarely caused by *Clostridium perfringens* type A, and has zoonotic risk as it can cause food poisoning in humans (Popescu, 2010).

Status of mastitis pathogens in India

A plethora of bacteria isolated and designated as etiological agents of mastitis in India are listed in Table 2. The *S. aureus* has been reported as the chief etiological agent of mastitis in India and in most of the Asian countries by various researchers (Rahman *et al*., 2010; Sharma and Maiti, 2010). Several studies reported S. *aureus* as the major pathogen in different parts of the country like Jammu (Sudhan *et al*., 2005), Maharashtra (Awandkar *et al*., 2009), Izatnagar (De & Reena Mukerjee, 2009) and Bangalore (Sumathi *et al*., 2008). The capsular typing of *S. aureus* from bovine mastitis revealed 60% of *S. aureus* were found to possess cap5K gene responsible for production of CP5 capsule and 20% of the isolates possessed cap8K gene responsible for biosynthesis of CP8 capsule (Upadhyay *et al*., 2010). Also the prevalence of CNS among bacterial isolates from milk samples increased from 9.91% in 2003 (Sharma and Prasad, 2002) to 72.13% in 2009 (Dutta, 2009). Among the staphylococci, predominance of *S. epidermidis* over *S. aureus* has been reported from Hisar, Haryana (Sharma *et al*., 2006). Thus, CNS is now emerging as a major pathogen associated with subclinical mastitis (Ahire *et al*., 2008). In a study to assess the prevalence of the mastitis pathogens by Shome *et al*. (2011), it was found that 74.04% of intramammary infections were due to *Staphylococcus* spp. including the coagulase negative staphylococci. The most predominant species was *S. aureus* (33.1%) followed by *S. chromogenes* (24.3%), *S. epidermidis* (16.7%), *S. sciuri* (9.6%), *S. haemolyticus* (7.9%), and other staphylococcus (8.4%) (Fig.1). In India, *E. coli* is also a common mastitis pathogen subsequent to *Staphylococcus* spp. Different studies in India report the prevalence of *E. coli* to be 14% forming the second major pathogen causing bovine mastitis (Kumar *et al*., 2010; Shome *et al*., 2011).

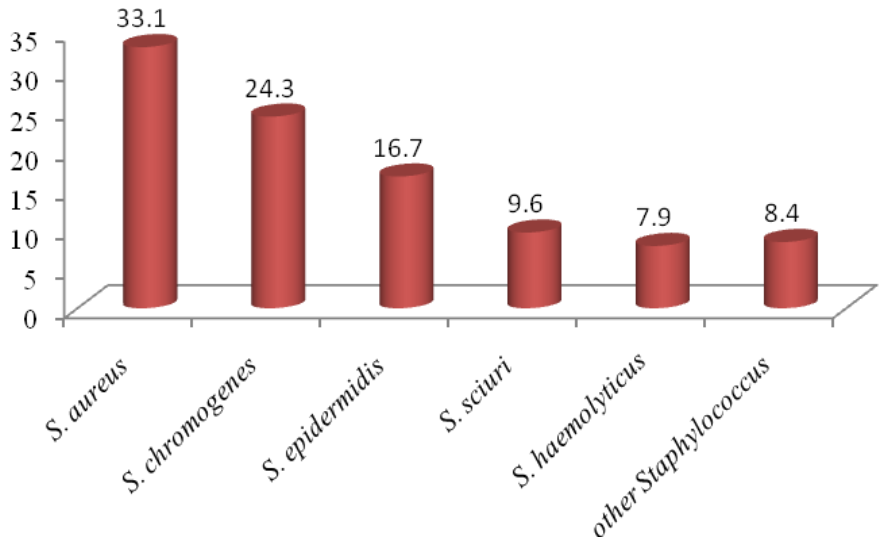

Fig.1:Prevalence of *Staphylococcus* spp. from bovine milk samples (Shome *et al.*, 2011)

Table 1: Distribution of common mastitis organisms in India

Prevalence (%) of common mastitis pathogens					Reference
S. aureus	**CNS**	**Strep. spp**	***E. coli***	**Others**	
74.71	-	21.13	-	4.15	Singh *et al* (1982)
42.10	-	5.26	18.95	3.16, 4.74, 5.26, 2.10	*et al*, (1993)
60.32	-	31.98	-	-	Shukla *et al*,(1998)
74.04	-	6.00	-	7.32, 2.93, 5.71	Patel *et al*,(2000)
39.01, 52.48	-	-	-	8.51	Ghose and Sharada (2003)
18.99	-	15.50	-	17.05	Jha *et al*, (2004)
27.90	16.28	6.98	17.44	5.81,4.65, 5.81, 4.56, 3.49, 3.49, 3.49	Das and Joseph (2005)
-	-	15.45	12.73	10	Sharma *et al*, (2007)
34.38	25	9.38	21.87	6.25, 3.12	Yathiraj *et al* (2007)
39.53	-	20.98	9.30	16.27, 6.97, 6.97	Vishwakarma (2008)
16.66	40.47	33.38	-	9.52	Ahire *et al*. (2008)
59.37, 4.90	-	16,10.63,1.1	2.9	2.2, 1.8, 1.1, 1.1, 1.1	Sahoo *et al*. (2009)
27.86	72.13	-	-	-	Dutta *et al*. (2009)
27.27, 15.91	-	50,4.55	-	2.27	Kumar *et al*.(2009)
57.27	-	15.45	12.73	10	Sharma and Maiti (2010)
27.37	12.63	5.79	8.95	7.89,1.35	Ranjan *et al*. (2011)
33.83	97.8	-	-	-	Krithiga *et al*. (2011)
33.14	49. 5	11.63	14.04	-	Shome *et al*. (2011)

The factors like herd size, agro-climatic conditions of the region, variations in socio-cultural practices, milk marketing, literacy level of the animal owner, system of feeding and management were found important affecting the incidence of subclinical mastitis (Joshi and Gokhale, 2006). However, overall prevalence of bovine mastitis in India is 44.67% (ranged from 25.63 to 97.61%). This data is calculated as mean of more than 100 studies of 21 states of the India. This range (25.63 to 97.61%) of bovine mastitis occurrence clearly indicates the drastic increase in the prevalence of mastitis.

Bovine mastitis: A threat to public health

Apart from its economic importance, mastitis is also a public health concern. Milk drawn from an infected cow can transmit pathogenic bacteria to humans through food chain which is dangerous to the public health. Milk can transmit tuberculosis, brucellosis, diphtheria, scarlet fever and Q fever to humans. However, these diseases can be controlled by a pasteurization technique, but a variety of bacteria still contribute to illness and diseases outbreak. Presence of bacterial toxins and antibiotic residues in the milk poses threat to human health. The milk

from an infected animal is the main source of pathogenic bacteria and some bacterial toxins produced in the milk cannot be destroyed by heating or drying. The heat-resistant enterotoxins produced by *S. aureus*, are responsible for staphylococcal food poisoning outbreaks. The presence of antibiotic residues in milk is mainly due to extensive use of antibiotics in the treatment and control of the disease. Antibiotic residues in foods can lead to severe reactions in people allergic to antibiotics and, at low levels, can cause sensitization of normal individuals and development of antibiotic-resistant strains of bacteria.

Antibiotic selection for mastitis treatment needs culture sensitivity tests which are not available in Indian field conditions. Indiscriminate and frequent use of these antibiotics in animals could be the reason for their ineffectiveness against bacterial isolates. Production of plasmids mediated beta-lactamase enzymes is supposed to be mainly responsible for resistance to penicillin. The evolution of antibiotic resistance in *S. aureus* strains is a serious cause of concern in dairy animals. Strains of *S. aureus* resistant to β-lactam antibiotics are known as methicillin-resistant *S. aureus* (MRSA). Studies suggest that people working with livestock are at potential risk of becoming carriers of methicillin resistant staphylococci, because of the spread of the emerging methicillin resistant strains between animal populations and human populations, although the direction of transfer could not be proven (Huber *et al.,* 2010). Especially, the detection of methicillin resistant *S. aureus* (MRSA) and CoNS (MRCNS) strains, in raw milk is regarded as an issue of great public health concern for their potential spread through the dairy food chain (Virdis et al., 2010). Also MRSA strains have been observed to be multi-drug resistant, such as aminoglycosides, macrolides, lincosamides, streptogramins, tetracyclines etc., which are often used in the treatment of mastitis (Kumar *et al.*, 2010).

A recent study in at PD_ADMAS, Bangalore revealed out of 696 staphylococcal isolates 2.73%, 0.43%, 0.43% and 2.01% from milk, extramammary site, animal environment and animal handlers showed methicillin resistance by exposing *mec*A gene and typeV SCC*mec* element was found to be the predominant among these resistant isolates (unpublished data). Even gram negative bacteria can produce enzymes like Extended spectrum β-lactamases (ESBLs) that can degrade and confer resistance to some of the most commonly used antibiotics including penicillins, cephalosporins and monobactams (Bonnet, 2004). Apart from ESBLs, resistance to carbapenems due to the production of metallo-beta-lactamases (MBLs) in gram negative organisms is increasing international public health problem. Recent studies describe the occurrence of ESBL-producing *Enterobacteriaceae* in raw milk, therefore, the impact of animal food chain as reservoirs and disseminator of such strains into must be assessed.

Conclusion

The significant increase in the occurrence of bovine mastitis is an alarming phase for the dairy sector. In spite of decades of research, mastitis still remains as a complex disease condition difficult to resolve due to the involvement of a wide range of etiological agents. Considering that different pathogens are the predominant cause of mastitis in different countries, mastitis controls can be developed to meet the specific requirements of an individual country or segment of the dairy industry. The advent of molecular biological techniques also has circumvented some of the problems associated with the conventional microbial procedures and gave promising option for the rapid detection of bacteria, even in cases of difficult-to-detect subclinical mastitis. Identifying the microorganisms responsible for culture negative, clinical mastitis and assessing changes in bacterial populations throughout infection will improve our understanding of the disease process and help us to

identify effective intervention strategies. Still, a more in depth knowledge of the etiological agents is crucial to gain better understanding of pathogenesis and epidemiology of bovine mastitis. Relatively little is known about the genetic basis for the virulence of the organism, their natural physiology including, factors that enable it to colonize and get established in the host. For these reasons, it is essential to learn as much as possible about the organism itself, including its complete repertoire of genes and overall genome organization. Further, genome analyses will lead to the discovery of novel genes, key virulence associated factors, and altered genomic organizations, which will give a better understanding of the molecular evolution of these pathogens. This in turn forms a rich source of unexplored areas for future hypothesis based research aiming to investigate their roles in pathogen virulence and disease pathogenesis. The results of such studies will provide the foundation from which researchers will be able to investigate the molecular basis of virulence of the pathogens causing bovine mastitis and will also help in identifying appropriate targets for the next generation of antimicrobial agents and vaccines to treat and prevent mastitis in dairy cattle. We hope, necessary attention is paid to this problem so that a better solution can be worked out to counter the problem of mastitis.

15. Importance of Laboratory Investigations in Epidemiological Studies

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There are many infections/diseases which may be transmitted through the veterinary hospitals/ dispensaries. This scenario is not different from hospital acquired infections in human beings. The animals brought for treatment / artificial insemination or any other purpose may/may not be clinically healthy. One common example is the animal brought for mastitis case. The mastitis case may be infectious/non-infectious origin. If it is infectious origin, the milk tested and discarded serve as source for infection to other animals. Rarely, some cases may be antibiotic resistant cases, then the situation becomes more dangerous for acquiring resistant organism and spread to new areas.

Same way, infections caused by diarrhoea organism's bacterial/viral /parasitic origin the samples collected in the clinics not disposed/disinfected properly will be acting as source of infection to others. The clinical microbiology laboratory is essential to a comprehensive infection prevention program. In such cases, the primary role is monitoring through which we can understanding the epidemiology of hospital transmitted diseases, determining rates of infection/reproduction rates of infection and Surveillance. The next role is to prevent - intervention to prevent infections, educating the staff and other people in hospitals. The third role is Control –outbreak investigation.

Sequential Steps involved in laboratory investigations
i) Specimen collection ii) Accurate identification and susceptibility testing
iii) Laboratory information systems iv) Rapid diagnostic testing
v) Reporting of Laboratory data vi) Outbreak Recognition and investigations –Molecular Typing vii) Organisms storage viii) Culture of Specimens from hospital staff and the environment

Things to be considered while collecting Specimens for investigation
The personnel collecting the specimen should be educated on proper specimen collection for intended diseases and transport medium/media used for different diseases. After the collection of specimens, quality has to be monitored. If the received specimens do not meet the prescribed quality, reject the improper specimens. Accurate identification of pathogens in the sample specimen is essential. Identify causative organisms rapidly and accurately to species level. There may be different types of samples received for identification /diagnosis.

Diagnostic samples in diseases/outbreak cases; Surveillance samples – eg: post operation surveillance plan samples (POSP) collected after Avian Influenza outbreak containment operations; Environment samples in toxicity suspected cases. There are expanding spectrum of organisms that colonize and infect seriously ill / immunocompromised challenges the ability to identify and characterizes pathogens accurately. The personnel involved in the laboratory diagnosis should have adequate knowledge on various diagnostic techniques and interpretation of the results. Educated and well trained personnel are required to provide accurate diagnosis. Molecular testing able to provide rapid diagnosis .Molecular testing will not be able to differentiate the samples from Live versus Dead. Hence the personnel working should have proper record of the samples. At the same time , the persons should be careful about cross reactivity. While processing the samples, the DNA/RNA should not cross

contaminate between the samples. Susceptibility testing of pathogens before treatment in special cases such as antibiotic no responsiveness. Performing accurate susceptibility testing is required in these cases. Since this is an antibiotic resistant era, continuous survey for MDR organisms required. Sometimes, unexpected antimicrobial resistance cases may show up.

Rapid Diagnostic testings: Central laboratories may be equipped with molecular diagnostics equipments which provide diagnosis within days-hours. These laboratories can look for panel of microbes in the samples depending on the symptoms/sample types

Laboratory Information Systems

The microbiology laboratory should choose a LIS System that can also be used by IC to data mine. The microbiology laboratory is an Early Warning system. A Laboratory Information Management System (LIMS), sometimes referred to as a Laboratory Information System (LIS) or Laboratory Management System (LMS), is a software-based laboratory and information management system with features that support a modern laboratory's operations. Key features include — but are not limited to —workflow and data tracking support, flexible architecture, and data exchange interfaces, which fully "support its use in regulated environments. The features and uses of a LIMS have evolved over the years from simple sample tracking to an enterprise resource planning tool that manages multiple aspects of laboratory informatics.

The LIMS is an evolving concept, with new features and functionality being added often. As laboratory demands change and technological progress continues, the functions of a LIMS will likely also change. Despite these changes, a LIMS tends to have a base set of functionality that defines it. That functionality can roughly be divided into five laboratory processing phases, with numerous software functions falling under each:

- the reception and log in of a sample and its associated customer data
- the assignment, scheduling, and tracking of the sample and the associated analytical workload
- the processing and quality control associated with the sample and the utilized equipment and inventory
- the storage of data associated with the sample analysis
- the inspection, approval, and compilation of the sample data for reporting and/or further analysis

There are several pieces of core functionality associated with these laboratory processing phases that tend to appear in most LIMS:

The core function of LIMS has traditionally been the management of samples. This typically is initiated when a sample is received in the laboratory, at which point the sample will be registered in the LIMS. Some LIMS will allow the customer to place an "order" for a sample directly to the LIMS at which point the sample is generated in an "unreceived" state. The processing could then include a step where the sample container is registered and sent to the customer for the sample to be taken and then returned to the lab. The registration process may involve accessioning the sample and producing barcodes to affix to the sample container. Various other parameters such as clinical or phenotypic information corresponding with the sample are also often recorded. The LIMS then tracks chain of custody as well as sample location. Location tracking usually involves assigning the sample to a particular freezer location, often down to the granular level of shelf, rack, box, row, and column. Other event tracking such as freeze and thaw cycles that a sample undergoes in the laboratory may be required.

Modern LIMS have implemented extensive configurability, as each laboratory's needs for tracking additional data points can vary widely. LIMS vendors cannot typically make assumptions about what these data tracking needs are, and therefore vendors must create LIMS that are adaptable to individual environments. LIMS users may also have regulatory concerns to comply with such as CLIA, HIPAA, GLP, and FDA specifications, affecting certain aspects of sample management in a LIMS solution. One key to compliance with many of these standards is audit logging of all changes to LIMS data, and in some cases a full electronic signature system is required for rigorous tracking of field-level changes to LIMS data. LIS alerts –which gives alerts on the various points. After appropriate diagnosis and interpretation of results, Data should be summarised and reported.

There are standard diagnostic techniques recommended by the organisations like WHO,OIE, FAO for each disease. The gold standard tests are mostly being used for testing the outbreaks. If the laboratory is not equipped with the required tests, can perform alternate tests with guarded results. Laboratory investigations play important role in epidemiological studies which includes outbreaks, surveillance, monitoring, surveys, etc. Laboratory investigations are important from providing accurate and timely diagnosis of the cause of outbreaks to disease control measures. The samples sent during any outbreak event or the suspected samples are actively investigated for prompt diagnosis and to speed up the control measures to contain the disease in a particular area/region. At the same time , the routine samples collected should also be investigated seriously to ensure not missing any emerging /re-emerging /new infections in the area. Apart from list of endemic diseases in a region, the results of routine diagnosis provides clues of any new endemicity of any other diseases in any species or the species jumping of the endemically known disease.

Molecular typing

Molecular typing methods determine the clusters of organisms, which subtype, genotype present in that geographical region , the association of clusters, transmission areas, patterns etc.

Organism storage

The organisms isolated should be properly stored for further supplemental testing. The laboratory and infection control need to decide which isolates should be frozen and for what period of time.

Cultures of Specimens from Hospital Personnel and the Environment

These cultures should be performed rarely and only when epidemiologically necessary. Detection of isolates does not determine cause of the disease.

As a whole, those laboratories working on investigations the work nature becomes increasingly complex and demanding because of continuous emergence and re-emergence of pathogens , newer technologies for diagnosis , increasing resistance in case of antibiotic resistance organisms. Their role becomes more vital in any epidemiological related studies.

16. Applications of GIS and Remote Sensing in Veterinary Epidemiology

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I. GIS in Veterinary Epidemiology

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 component 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.

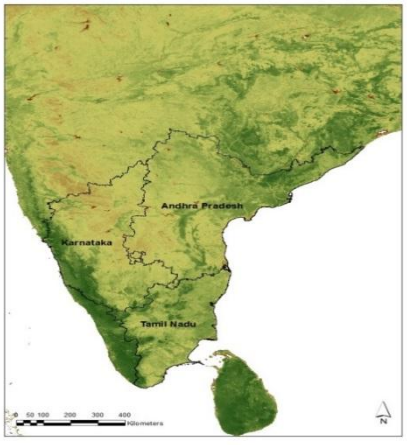

Figure 1: Satellite image in the raster format showing vegetation in South India.

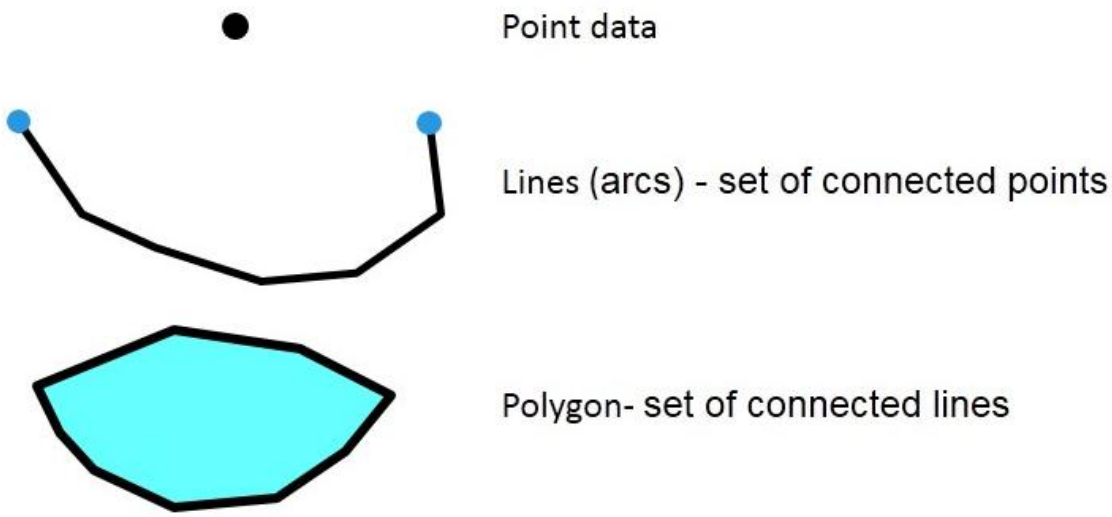

Figure 2: Types of vector data – point data, lines and polygon

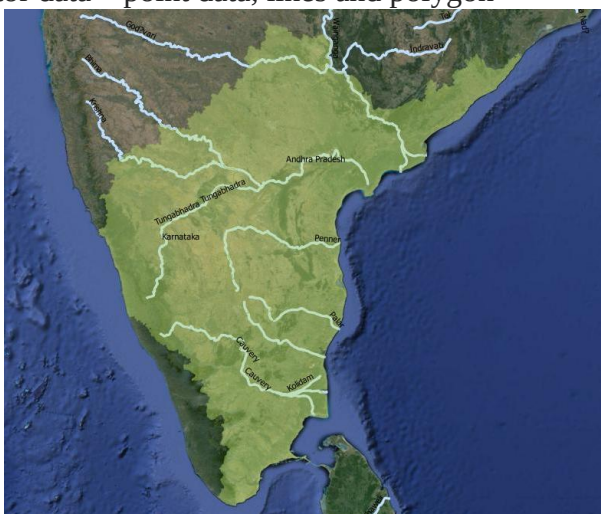

Figure 3: Line map showing the major rivers flowing through South India

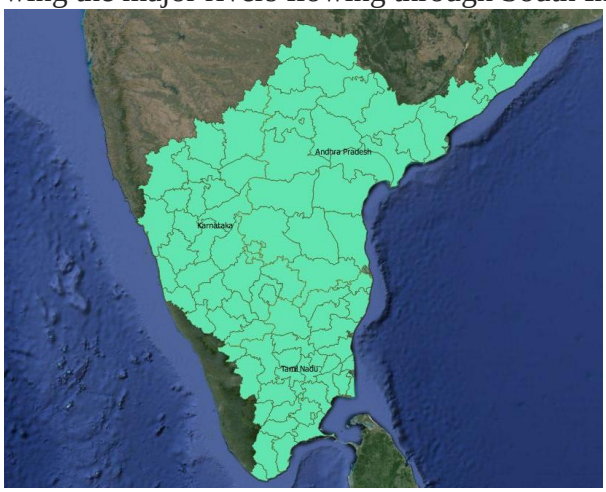

Figure 4: Polygon map showing the different districts of three South Indian states.

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).

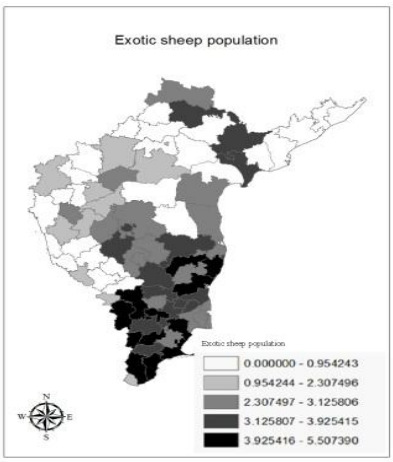

Figure 6: The Choropleth map showing the population of Exotic and crossbred sheep in three South Indian states. (Darker the colour more the density)

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).

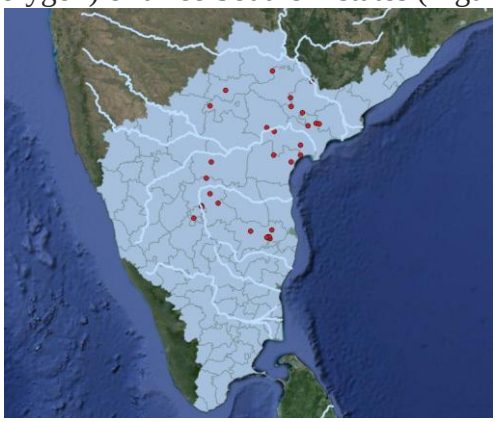

Figure 7: Over layering of different spatial data. The dots (Point map) indicate location of the outbreaks, line (line map) indicates the rivers flowing near the outbreaks regions and the districts (polygon map) shows the location of districts with respect to the outbreaks location.

5. **Disease modelling:** 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 a 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 modelling 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 modelling 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 modelling and forecasting of diseases of public health importance (Rogers, Randolph et al. 2002). The use of remotely sensed variables in developing risk maps and modelling 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 modelling 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.

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17. Epidemiology of Haemorrhagic Septicemia and Anthrax

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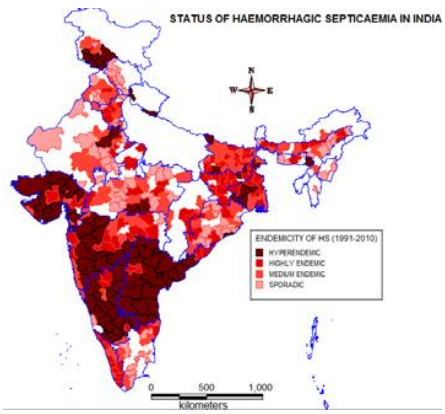
India has been the major country in Asia as well as in world with highest domestic animals population including cattle (185 million) and buffaloes (98 million), which are reared mainly for use as draught animals as well as milk production in the region. However, health scenario of cattle and buffaloes are threatened by occurrence of deadly diseases. Among bacterial diseases, haemorrhagic septicaemia (HS) and anthrax outbreaks are of economic importance in India. It is commonly believed that the reported number of HS and anthrax outbreaks and affected cases represent only a fraction of the actual number in India. The available reports indicating the number of outbreaks, morbidity and mortality statistics of HS and anthrax from different parts of the country are hardly complete. With the existing sources of information, it would be a great challenge to assess the actual prevalence and distribution of HS as well as anthrax to estimate the realistic economic losses in different regions. It also causes difficulty in studying the epidemiological characteristics with a view to formulating and implementing effective methods of control and prevention. Although, majority of animals treated with broad specturm antibiotics following analysis of antibiotic sensitivity pattern, in early stage of infection, before toxaemia/bacterimia are effective, with the emergence of drug resistance bacterial strains the incidence of disease outbreaks has increased. Nevertheless, concerted nationwide efforts are required to assess the comprehensive epidemiological aspects of HS and anthrax outbreaks in India.

Epidemiology of HS in India

Haemorrhagic septicaemia (HS), is an acute, fatal and septicaemic disease of cattle and buffaloes caused by bacterium *Pasteurella multocida,* has been recorded in almost all geographical regions of India. The different serotypes viz., capsular (A, B, D, E, F) and somatic (1-16) of *P. multocida* are associated with a variety of disease/syndrome, generally termed as 'Pasteurellosis', in wide range of domestic as well as wild animals/birds. In many instances, *P. multocida* plays a secondary role in the pathogenesis of the disease or acts in combination with other bacterial, viral or parasitic infections. HS, which is a primary pasteurellosis with 100% mortality in infected animals, is endemic in most parts of India, which ranks HS as one of the most economically important bacterial disease. It is estimated that, from 1936 to 1944, there were an average of 700 reported outbreaks of HS and 40,000 deaths per year. The average high mortality per year ranged from 30,000 to 60,000 until 1960s, however, the death rate started to decline to approximately 4000 per year from 1970s, presumably due to improved control measures in the region (FAO, 1991). It has been reported that during the past four decades HS has accounted for 46-55% of all bovine deaths. Following the successful control or eradication of RP and continued low mortality with FMD, HS has emerged as a disease of considerable economic importance in India. In India, typically *P. multocida* serotype B:2 is involved in cases of HS among cattle and buffalos. However, the previous investigations of field isolates revealed presence of other serotypes such as A:1, A:3, A:4, A:1,3, F:3, F:3,4 and F:4,12 especially serotype A:1 as next



predominant to type B:2. A considerable variation among serotypes with respect to host predilection, pathogenicity, biochemical, cultural and antigenic specificity has been reported.

The disease is known to occur in acute, subacute and chronic forms. The disease transmission is through direct contact with infected/carrier animal or contaminated materials in the premises as well as movement of animals and/or personnel. The distribution and frequency of disease occurrence has been attributed to climatic conditions, husbandry practices and types of animals reared. The HS outbreaks are known to occur throughout the year irrespective of the season as per the known perception that the disease is mostly precipitated during the monsoon or post monsoon period. However, the incidences of disease outbreaks in India are known to vary considerably in different states and also in each state from year to year. the infection is generally caused by several strains/variants of specific serotype of *P. multocida* B:2. It is prevalent mostly in the regions, where husbandry practices are poor. Incidence and distribution of HS varies greatly from a few cases to high number reports depending on the geographical area and agro-climatic conditions prevailing in various regions of the country. Buffaloes are generally believed to be more susceptible to HS than cattle, and in this species, the disease course is shorter. In endemic areas, most deaths are confined to older calves and young adults. In non-endemic areas, massive epizootics may occur. Case fatality approaches to 100%, if treatment is not carried at early stage. The presence of naturally acquired immunity is observed among buffaloes. It has been opined that HS morbidity and mortality pattern, in endemic and non-endemic areas in a given population, is largely dependent on the proportion of naturally acquired immune to non-immune animals.The presence of HS-causing pasteurellae in small proportion in the naso-pharynx of apparently normal healthy cattle and buffaloes and recent exposure to outbreaks of HS has been documented for a long time. Further investigations, in experimentally exposed buffaloes and induced carrier animals which remained clinically normal, showed the persistence of pasteurellae in the crypts of tonsils for several months despite treatment with antibiotics, before they appeared actively in the nasopharynx and shed the organisms intermittently in the nasal secretions. The factors, responsible for influencing the latent carriers to become active, are still an enigmatic. It would appear that the extent of rainfall and its geographic and temporal distribution are important factors influencing the incidence and change from enzootic to epizootic occurrence of HS. The annual fluctuations in the incidence are difficult to interpret. Other factors known to influence the occurrence and spread of HS include the mean air temperature, relative humidity, parasitic infections, carrier rate of *P. multocida* and herd immunity level etc. It is always a great challenge to predict the unpredictable environmental factors that kick off the disease outbreaks. Moreover, the occurrences of HS outbreaks despite vaccination generate a new set of questions which remain unanswered because neither the precise mechanism of immunity nor the specific bacterial antigens involved in immunity to HS have been clearly identified so far. Overall, occurrence of the cases of HS has, however, shown a declining trend in India. Nevertheless, there is an imminent call for thorough investigation and reporting of each and every outbreak of HS from remote areas of the states to fill up the lacunae in the present data, because the reporting of HS is quite inadequate to launch an effective control strategy.

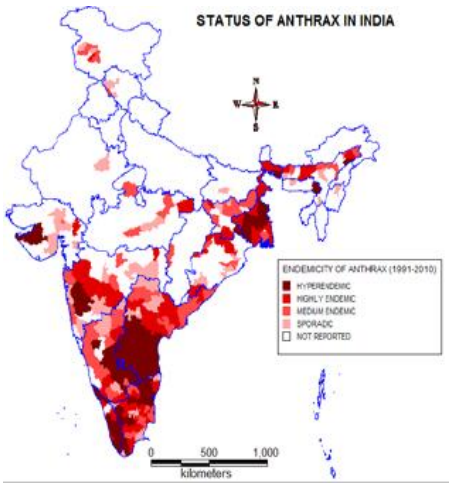
Epidemiology of anthrax in India

Anthrax (synonyms are ‘charbon’, ‘wool sorters disease’, ‘rag pickers disease’, malignant carbuncle, and ‘malignant pustule’), is an acute and fatal bacterial disease, derives its name from the Greek word for coal – *anthrakis*, as it causes black coal like skin lesions. Caused by *Bacillus anthracis* organisms, the disease is known to affect herbivores predominantly, which is transmissible to humans. Since, the earliest historical records until the development of an effective veterinary vaccine (Sterne 1937), together with the

subsequent development of effective antibiotics, the disease has been one of the foremost causes of uncontrolled mortality in herbivores (cattle, sheep, goats, horses and pigs) worldwide. Humans are almost invariably known to contract anthrax directly or indirectly from domestic animals. In recent times, the disease has received an exceptional global attention mainly due to the possible use of bacillus spores in biological warfare and bioterrorism. Anthrax is an archetype anthropozoonosis which affects a wide range of animal species, especially herbivorous mammals, and is transmissible to humans through touching either animals killed by anthrax or infected products. Increased international trade in animals and animal products, as well as intense travel of people around the world, represent greater risks of dissemination of anthrax with a higher devastating global impact on public as well as livestock health scenario. Anthrax has worldwide distribution and has been reported from every country having livestock population. The disease is most prevalent in tropical and subtropical countries which practice largely pastoral agriculture. From the global disease prevalence data reported to the OIE, it is evident that anthrax is enzootic in most countries of Africa and Asia, including India. In many other countries, the disease occurs sporadically. Despite the global prevalence of anthrax, in many cases of incidences of disease outbreaks go unreported.

The causative agent of anthrax, *Bacillus anthracis*, is a obligate bacterial microbe. Its vegetative forms are more 'fragile' and die more spontaneously in simple environments such as water or milk. The organism, which is in the vegetative form inside the infected host, when released into the outside environment ceases its multiplication under adverse conditions and undergoes sporulation in the presence of free oxygen to form spores. The spores are markedly resistant to environmental extremes such as heat, cold, pH, and desiccation etc. as well as to chemicals including disinfectants, irradiation, and other extreme conditions. Hence, spores constitute the predominant phase of the organism in the environment and can remain there for a longer duration. The spores germinate within the infected host to produce vegetative forms which multiply, eventually killing the host. Climate probably acts directly or indirectly by influencing the way in which an animal comes into contact with the spores during close grazing or water scarcity or varied level of host resistance to infection.

Anthrax continues to be endemic in most parts of India, where animal husbandry practices are poor. The disease is enzootic in southern India but it is less frequent to absent in the northern states where soil is more acidic. Many factors such as poor disease surveillance coverage, inadequate diagnostic capacity, and lack of clear strategies to address the plight of zoonotic diseases have been contributing to under-diagnosis and under-reporting of zoonotic diseases. In India, the incidence of anthrax is known to vary greatly in various agro-climatic zones of the country, with widely reported incidences in the south zone followed by east, west and northeast zones. The data of the National Animal Diseases Referral Expert System (NADRES) of the PDADMAS for the last two decades (1991-2010), anthrax features as one of the top ten diseases reported in India and also a major cause of deaths in livestock. Anthrax has been reported in eighteen states of India especially in southern states. There has been year to year variation in disease reporting/occurrence. The epidemics of anthrax are known to increase in some seasons especially between July to September and in November and January, coinciding with the post monsoon months across the country. Seasonal variation in anthrax occurrence has also been reported between the different zones of India. Mortality due to anthrax can be very high in



herbivores. The susceptibility to anthrax varies considerably among different animal species. Naturally, herbivores are particularly susceptible, and omnivores and carnivores are moderately resistant but still succumb. Generally, higher incidences have been reported in large ruminants (cattle and buffaloes), followed by small ruminants (sheep and goat) and a fewer outbreaks reported in pigs and elephants. Although reports of anthrax occurrence in dogs/carnivorous animals wild animals scavenging anthrax carcasses have been reported from zoological gardens and wildlife sanctuaries or national parks, outbreaks affecting large numbers of carnivorous animals are very rare. Although, the disease has been recognized for centuries, it has not yet been established scientifically on how grazing healthy animals contract it. Several predisposing factors such as season, rainfall, temperature, soil, vegetation, host condition and population density are considered on the epidemiology of anthrax. Some reports indicate that anthrax outbreaks are likely to develop mainly during the dry months that follow a prolonged period of rain when the spores are exposed and the ruminants have greater access to them. Hot and humid season facilitates germination of spores in the environment. However, there is a need to understand the multi-factorial role in persistence and precipitation of anthrax outbreaks.

Conclusions

Insufficient data on incidence, occurrence and distribution of HS and anthrax coupled with inefficient prophylactic and control measures make an insidious impact on the livestock sector of India, drastically affecting the our growing economy that solely depend on agriculture, livestock and allied sector with an overall contribution of 28-32% to the nation's GDP. The need of the hour is to focus on the key thrust areas such as strengthening the national disease monitoring and surveillance system, networking of well-equipped remote/regional laboratories with easy accessibility to endemic areas, supporting the region based applied research, effective implementation of national mass prophylactic vaccination programme, training of veterinarians about recent advances in rapid disease diagnosis/treatment/control measures as per the international standards (OIE 2008) and mass awareness programmes focused at advanced animal husbandry practices by multi-pronged extension activities at rural level which would ultimately help in control n of HS and anthrax in India.

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18. Epidemiology of Parasitic Diseases in India

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It is well said by Elton, the ecologist that ' A parasite's existence is usually an elaborate compromise between extracting sufficient nourishment to maintain and propagate itself and not impairing too much the vitality or reducing the numbers of its host, which is providing it with a home and free ride". In most of the parasites, life cycle consists of two phases, first phase is within its definitive host and the second phase is either within intermediate host/vectors or in the environment as free living stages. So in order to successfully complete its life, a parasite must adjust its way of living in these two entirely different environmental conditions, namely that provided by the host and that prevailing in the non-parasitic habitat. This inturn will determine the epidemiology of any parasitic disease. Epidemiology is not only concerned with the distribution pattern of a disease in a population, but also deals with the risk factors/determinants that contribute to the occurrence of the disease as well as the application of this knowledge for the effective control 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 (or intermediate host or vector) which are generally determined genetically. Extrinsic determinants are normally associated with some form of environmental influence on the host or disease agent (or intermediate host or vector). For parasitic diseases, epidemiology will depend upon the particular lifecycle pattern of each parasite, availability of vectors/intermediate hosts/ availability of suitable environmental condition, immune status of the host, managemental conditions, environmental changes, ecological disturbances etc. The frequency with which a disease occur in a population will depend on a large number of these determinants especially extrinsic determinants which may vary with space and time which means that the disease is a dynamic process. The type and pattern of diseases in livestock differ from country to country, area to area, species to species and production system to production system. The effective control of disease depends as much on a thorough understanding of the many complex factors that govern the changes taking place in a disease process. Considering these factors a generalized pattern of parasitic diseases can be discussed as below;

1. Epidemiology of trematode infections
2. Epidemiology of cestode infections
3. Epidemiology of nematode infections
4. Epidemiology of protozoan diseases

Epidemiology of trematode infections

In the life cycle of most of the digenetic trematodes, snails invariably act as the first intermediate host. Second intermediate hosts if it is present, may be snail/fish/ants etc. Thus the prevalence of trematode infection is not only influenced by the mere abundance of susceptible animals but also by the availability and efficiency of intermediate hosts as well as the grazing habitat of definitive hosts. Among the trematode infections, amphistomosis, fasciolosis and schistosomosis are predominant in ruminants in India. Optimum temperature, humidity and rainfall prevailing in India favours the survival of snail intermediate hosts as well as the developmental stages of the parasite since there is high distribution of wet/swampy grazing areas in India. For example; a study conducted by Fatima *et al*., 2012 in

Kashmir valley found that age and season are the most important risk factors for fasciolosis in cattle. During the wet season there is abundant grazing and alternate sources of drinking water. But during summer months there is a need for animals to graze near and to drink from available water sources where the risk of getting infection is more since snails will be also concentrated on to same water bodies. This will increase the chances of getting infected by the animals.

Epidemiology of cestode infections

Cestodes in ruminants can be classified into two distinct groups; one in which ruminants act as the final host (the intestinal and hepatic cestodes) and one in which cattle, buffaloes sheep and goats act as the intermediate hosts for the larval stages (Cysticercus, Coenurus and hydatid cysts) of various tapeworm species. In the latter group, the adult parasites live in the small intestines of domesticated and wild carnivores (*Taenia ovis, T. hydatigena*, *T. multiceps*, *Echinococcus granulosus*) and man (*T. saginata*).

Intestinal tapeworms include genera like *Moniezia, Thysaniezia* and *Avetellina* and hepatic tapeworm include *Stilesia hepatica*. The life cycles of these tapeworms are indirect and herbage mites of the family Oribatidae act as intermediate hosts. The mites, which are soil-inhabiting, surface during the night and early morning to feed on manure. During their feeding they accidentally ingest eggs of the intestinal tapeworms present in the manure, and the larval stage called a cysticercoid develops in the mites. Ruminants become infected by ingesting herbage containing mites carrying the infective stage of the parasite. In the case of disease where ruminants acts as intermediate hosts like bovine cysticercosis, poor standards of personal hygiene of infected human populations is responsible for the spread of disease. In some societies such as nomadic pastoral people there is a high risk of animals becoming exposed to infected faeces. Abnormal eating habits of cattle due to certain mineral deficiencies (pica) may result in cravings that increase the exposure through the ingestion of faeces. The survival of the eggs is strongly influenced by climatic conditions. Under wet and moist conditions, eggs may survive for months, exposing animals to a source of infection for a prolonged period of time. Eggs are very susceptible to dry conditions and are rapidly destroyed during the dry season.

Epidemiology of nematode infections

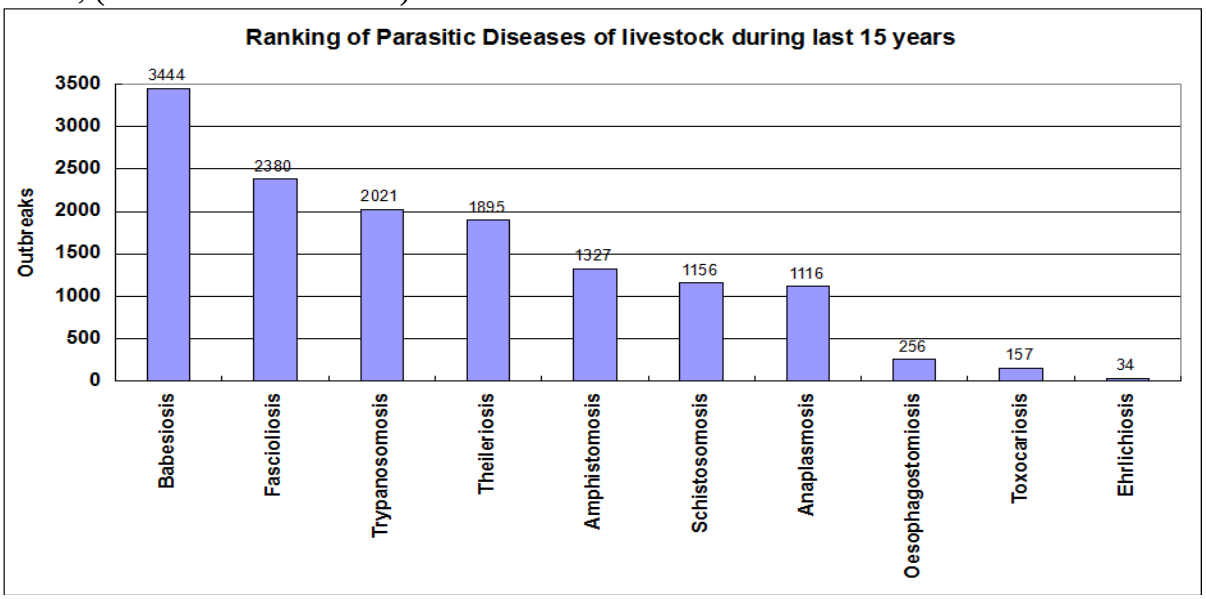
The most important and widely prevalent nematodes are the Trichostrongyle group (*Haemonchus, Ostertagia, Trichostrongylus, Mecistocirrus, Cooperia* and *Nematodirus*), *Oesophagostomum* and *Bunostomum.* The life cycle of these parasites are direct. Adult female nematodes produce eggs. The period between the infection of an animal by ingestion of infective L3 larvae and the first egg production by the adult female parasite is called the prepatent period. The development of larvae in the environment depends upon warm temperature and adequate moisture. In most tropical and sub-tropical countries, temperatures are permanently favourable for larval development in the environment. The ideal temperature for larval development of many species in the microclimate of the tuft of grass or vegetation is between 22 and 26 °C. The survival of larvae in the environment depends upon adequate moisture and shade. Desiccation from lack of rainfall kills eggs and larvae rapidly and is the most lethal of all climatic factors. Larvae may be protected from desiccation for a time by the crust of the faecal pat in which they lie or by migrating into the soil. Infective larvae may survive for up to 6 weeks or even longer in the manure pats, which act as a reservoir of infections during dry periods. The development of infective larvae ingested by an animal during adverse environmental conditions may be temporarily arrested in the abomasal or intestinal mucosa. This suspension of development helps some nematode parasites survive the dry seasons. Of the three larval stages in the environment (L1, L2, and L3) it is the L3

which has a protective sheath, that is the most resistant to variations in moisture, temperature and sunlight. Based on a nationwide survey on parasites epidemiology in dairy animals in seven different agroclimatic zones, Sanyal and Singh (1995) also indicated an increased parasite burden in the host and on pasture during the rainy seasons, the months for which vary in different agro climates.

Epidemiology of protozoan diseases

Distribution of protozoan diseases depends upon the availability of vector population in the environment. For example, Trypanosomes are mainly transmitted by Tabanus flies. The abundance of Tabanus fly population is depending upon the climatic conditions. Most suitable season for occurrence of trypanosomosis is during or after one and half to two months of rains due to availability of rain water lodged ditches for breeding of the flies (Ruprah, 1985). On the other hand coccidian parasites has got a direct life cycle and the survival and sporulation of the oocysts is facilitated by high humidity and moderate temperature.

Outbreaks of major parasitic diseases that occurred during the past 15 years are shown below; (Source: NADRES data)



Conclusions

Epidemiology of most of the parasitic diseases is affected by the surrounding climatic conditions. So for effective control of parasitic diseases, all these factors that contribute to the occurrence of the disease should be taken into consideration. A thorough understanding of this aspect will pave a way to prevent the occurrence of diseases in animals.

Suggested Readings

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19. Diagnostic Tests in Epidemiology

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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 a person 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)lI [b/(b + d)]

The LR+ is therefore a quantitative indication of the strength of a positive result. The per-fect 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) l! [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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20. Vaccine Epidemiology

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Vaccine is an agent specific antigen formulation with or without adjuvant that protects animal against specific disease by inducing specific immune response which lasts for varied time period depending upon nature of the antigen. Routine vaccination is used worldwide to control and prevent number of infectious animal diseases. There are various factors that influence the vaccine's ability to protect animal against disease (Fig.2). Hence, vaccine epidemiology gains importance as it leads to vaccine efficacy evaluation followed by identification and quantification of factors that influence vaccine efficacy and safety (Fig.1).

Given the scale of vaccination carried out annually and its implications in terms of animal health and economics it is essential to evaluate the field performance of the vaccine. Unlike human vaccines veterinary vaccines are not evaluated systematically for their efficacy in the field. Hence this article highlights the significance of vaccine evaluation in the field and methods to do it.

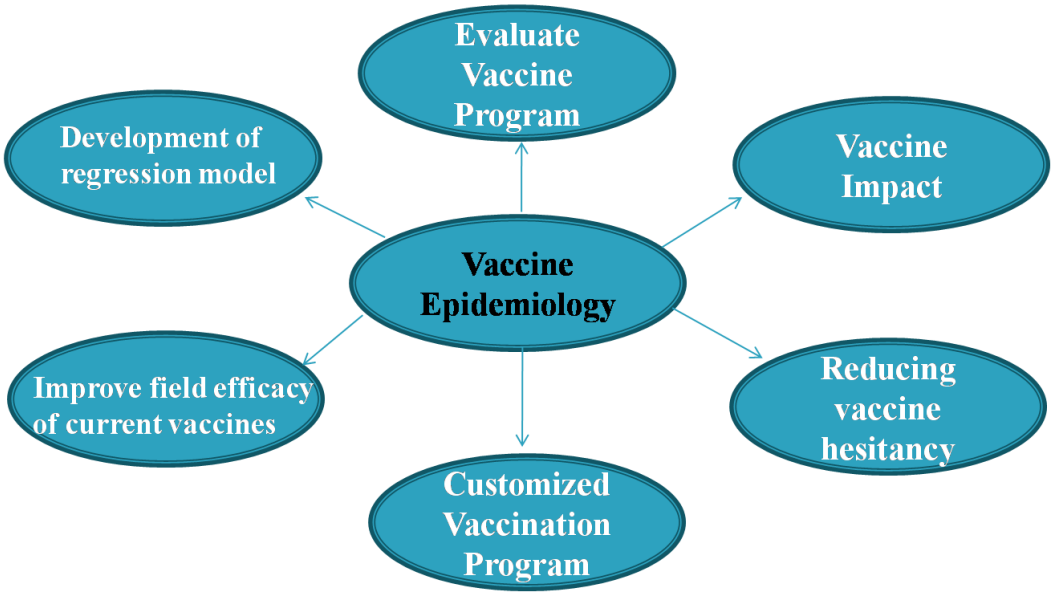

Fig.1 Applications of vaccine epidemiology studies

Vaccine Efficacy and Effectiveness

Vaccine efficacy of a vaccine is its ability to protect against the disease. There are number of factors that influence vaccine efficacy at different levels of vaccine formulation, manufacture, storage, supply and finally the end use. Newly developed vaccines are tested for their ability to protect against the challenge under laboratory conditions before they are released for field use. Further, the host immune response to the vaccine antigen is analyzed by evaluating the immune correlates of humoral and cell mediated immune response. Hence, challenge studies and sero-conversions are commonly used methods to establish efficacy of veterinary vaccines.

Though establishing vaccine efficacy at laboratory condition is a primary requirement but establishing the same at field condition will define the vaccine effectiveness (VE). Vaccine effectiveness is the proportion of vaccinated animals protected by the vaccine

against a defined outcome under field conditions. A given vaccine might have high or low VE but identifying reason for such variation is important to improve the existing vaccines. Hence in vaccine epidemiological studies calculating vaccine specific VE and factors that influence VE is utmost important (Fig.2). In rest of the paper focus is on VE and factors influencing VE.

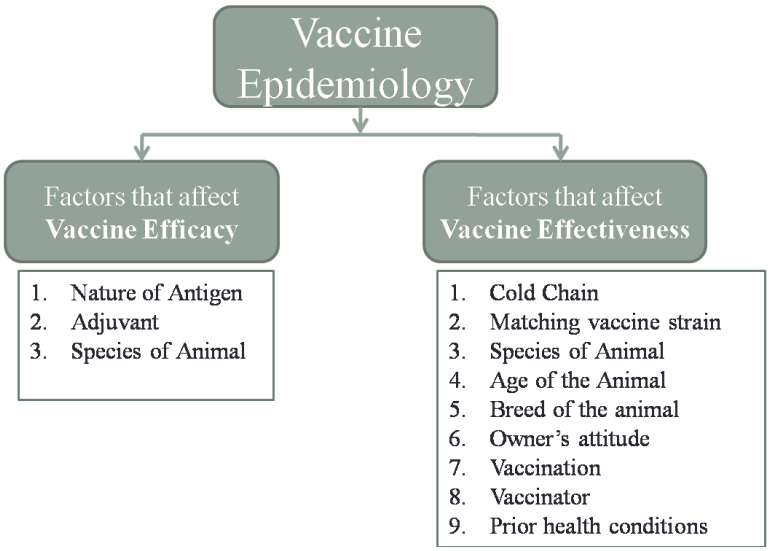

Fig.2 Factors influencing vaccine efficacy and vaccine effectiveness

Study Designs to Evaluate VE

There are study designs for evaluating various effects of vaccination and vaccination programs based on the choice of comparison groups, the unit of observation, the choice of parameter, and the level of information required. The most commonly used observational studies to evaluate the vaccine efficacy at field level include cohort study, case-control study and cross-sectional study.

Cohort study: A study design where vaccinated and unvaccinated samples (called cohorts) are followed prospectively and observed for occurrence of the disease (Fig.3). As the study is conducted, outcome from each animal in each cohort is measured and relationship with status of vaccination is determined. This study design has advantage of matching the subject cohorts which reduces the influence of confounding variables. The major disadvantage is difficult to identify cohorts due to confounding variables.

Case-control study: A study that compares the number of cases and controls in vaccinated and unvaccinated population (Fig.3). This study design looks back retrospectively to compare how frequently the vaccination of susceptible population is associated with protection from disease. These studies are mainly designed to estimate odds. The major advantage of this study approach is less time consuming and allows to look for multiple factors that can influence the vaccine efficacy at field level. But the disadvantage is retrospective studies depend on the event that has occurred hence data quality relies on memory. It also can be difficult to find a suitable control group for this study.

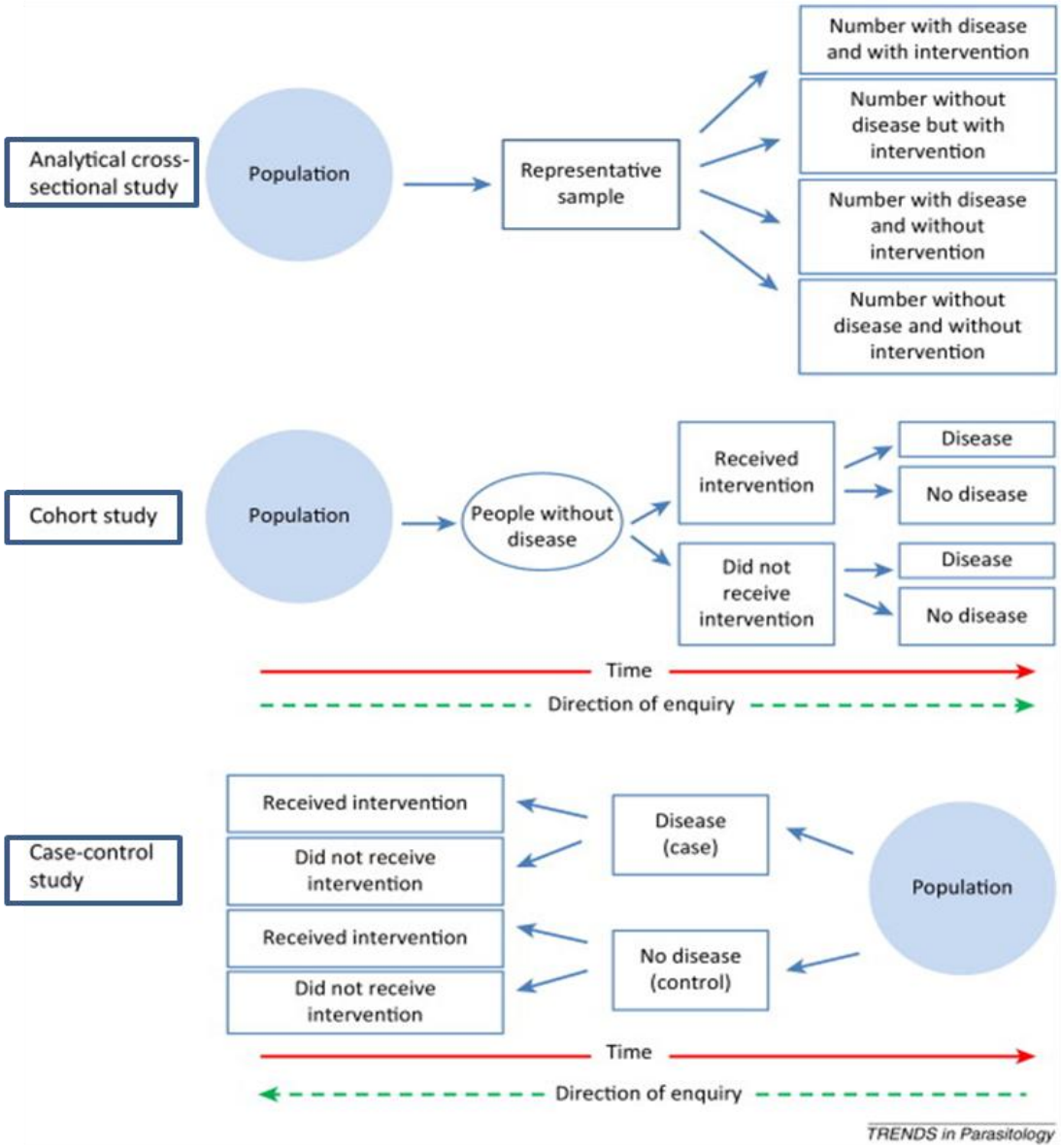

Fig.3 Study designs to evaluate vaccine effectiveness

Cross-sectional study: Cross-sectional studies are carried out at one time point or over a short period (Figure 3). They are usually conducted to estimate the vaccine effectiveness of the vaccine for a given population, commonly for the pilot study to design full vaccine effectiveness study. Hence, cross-sectional studies provide a 'snapshot' of the disease outcome in vaccinated/ unvaccinated population at a specific point in time.

Calculation of VE

A susceptible population divided in to vaccinated and unvaccinated. Such categories are kept under observation or retrospectively the cases and control data from both the groups are collected. A 2X2 contingency table is drawn with vaccinated/un-vaccinated verses cases/controls as shown in the Table.1. Further, using the formula the VE will be calculated.

Table.1 A 2X2 contingency table to calculate the effect of vaccination on disease occurrence

Exposure/ outcome	Cases	Control	Total
Vaccinated	a	b	a+b
Un-vaccinated	c	d	c+d
Total	a+c	b+d	a+b+c+d

$$\textbf{Vaccine Effectiveness (\%)} = \frac{\text{(ARU-ARV) X100}}{\text{ARU}}$$

ARU: Attack Rate in Un-vaccinated
ARV: Attack Rate in Vaccinated

Conclusion

Immunity of livestock population against specific disease can be achieved by potent vaccine and effective vaccination program. Hence evaluation of vaccine before and after the launch is critical. There are many factors which affect the vaccine efficacy at field level. Identifying those factors and quantification will help to decide the vaccine's ability to prevent disease and it further helps to predict the vaccine efficacy based on prevailing field conditions.

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21. Impact of Livestock Diseases - An Introduction

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Livestock health economics is developing as a new discipline and is progressively developing a solid framework of concepts, procedures and data to support the decision-making process in optimizing animal health management. Research in this field primarily deals with three interrelated aspects, viz. quantifying the economic impact of animal disease; developing methods for optimizing decisions when individual animals, herds or populations are affected; and determining the profitability of specific disease control and health management programmes.

The productivity of livestock is influenced by many factors such as nutrition, animal health, genetic potential, environmental condition and herd management. The constraints do not act independently on the system. After nutrition, animal health tends to be the most serious impediment to production. Livestock development programmes cannot succeed unless a well-organized animal health service is built up and protection of livestock against diseases and pests particularly against the deadly infections is assured. Any programme of animal health care needs certain information before the programme is started for its *ex-ante* analysis, when the programme is already in operation for mid-term appraisal and when the programme is completed for *ex-post* analysis. These information on animal health care help in taking decisions regarding the programme, execution and evaluation. The information available on the proportion of the livestock diseased over time and space and the extent of economic losses due to diseases in livestock are very scanty and in some cases it is not known at all.

Animal disease outbreaks are a significant economic threat to the livestock sector because the impacts of an outbreak can be quite costly and far-reaching. Any disease outbreak results in all the stakeholders comprising consumers, livestock producers, and allied industries sharing the economic impacts of animal disease and it is very complex to estimate the size of such impacts. From a public perspective, policy makers seek an accurate assessment of losses due to animal disease when weighing disease prevention and mitigation alternatives. Immediate impacts of a disease outbreak include a reduction in the productive capacity of the animal products industry and a subsequent reduction in the supply of livestock products. At the same time, disease outbreaks may reduce the demand for these products even from healthy animals in the infected area. Allied agribusinesses bear an initial loss in the supply of livestock products, and later, increased costs when locating and certifying safe food supplies also.

Challenges

The consequences of animal diseases in livestock and domesticated birds can be complex and generally go well beyond the immediate effects on affected producers. These diseases have numerous impacts, including:

- Productivity losses for the livestock (e.g. production losses, cost of treatment, market disturbances)
- Loss of income from activities using animal resources (in such sectors as agriculture, transportation and tourism)
- Loss of well-being of human beings (morbidity and even mortality rates, food safety and quality)
- Prevention or control costs (production costs, public expenditure)
- Suboptimal use of production potential (animal species, genetics, livestock practices)

These economic and social effects can be classified as 'direct', 'ripple' (impact on the industry's upstream and downstream activities), 'spillover' (impact on other sectors),'long term' or 'remote'.

Direct effects

The most direct economic impact of animal diseases is loss of production and/or productivity, and ensuring income losses for farmers. If the farm economy is diversified or if there are other opportunities to generate income, the impacts can be mitigated. However, if the economy depends on one or some of the vulnerable products, the impacts can be serious, and local food security can be threatened. The economic impact also depends on response strategies adopted by farmers and possible market adjustments.

Ripple effects

The livestock sector plays a significant role in the economic development of many countries. The production of meat and other animal-based food items generates income, jobs, and foreign exchange for all stakeholders in the animal industries. Consequently, an epizootic can affect the industry's upstream (inputs, genetic resources) and downstream activities (slaughter houses, processing and marketing) in terms of jobs, income for the stakeholders in the industry, or market access.

Spillover effects

Animal diseases can have major effects on food availability and quality for poor communities. It is well known that agriculture plays an important role in the generation of income and jobs in other sectors but the closeness of this interdependence became particularly obvious during recent epizootics of Avian Influenza in our country. The losses are difficult to calculate and would undoubtedly be much more significant in light of the extremely high mortality rates in developing countries.

Long term effects

It is difficult to calculate the cost of the public's loss of confidence in animal industries in their countries, or of an importer country towards the animal health care services of the exporting country. The loss of confidence by an importer can trigger a lasting embargo and major economic and social repercussions in the exporting country.

The major challenge in assessing the actual impact of animal diseases at any level is the lack of understanding and clarity on the part of the researcher to decide about the parameters to be

estimated which are generally grouped as direct and indirect impact indicators. They vary from disease to disease, and from species to species. Hence, so single or standard approach would suffice for the purpose.

Economic impact of animal diseases can be divided into six major parts, viz. production effects, market and price effects, trade effects, impacts on food security and nutrition, human health and environment, and financial costs. The immediate disease shock receives the most direct attention in impact studies because most often, it originates at the production phase. Yet, more far-reaching factors are sometimes overlooked. Disease impacts are generally easy to identify but may be difficult to quantify. In livestock, for example, delays in reproduction result in fewer offspring, which has long term effects not easily measured in the present state. Even though the disease can be managed optimally by private producers when the perceived economic damage is high, some level of disease if often accepted by managers when control is sufficiently costly. Still, researcher concludes that producer incentives for disease management can be changed through new technologies that lower the cost of prevention or control, subsidies or cost sharing of control measures, or on the consumer side, a change in public desire for disease risk-free products that changes relative prices. In short, livestock industry leaders would agree that disease outbreaks often have broader, long-term multiplier effects that extend beyond principal markets. Understanding the extent of such effects is an important element in measuring the potential costs and benefits of public policy tools to manage animal disease.The next major challenge at present is availability of quality data at specified time in our country. The present disease reporting system and database management are very imperfect and requires better understanding among the field veterinarians as they fear of negative implications for their service. Every effort should be made to convince the usefulness of economic impact studies, as they will be beneficial for their very existence and recognition from the policy makers. The most important challenge in conducting the economic impact of animal diseases as well as the forecasting animal disease outbreaks are the lack of sufficient qualified experts in the disciplines of livestock economics and statistics in our country. Hence, it is imperative on the part of us to develop expertise in taking up such studies on a continuous basis with respect to all major endemic animal diseases as well as emerging and exotic diseases.

Data sources

Two main sources of data on animal disease aspects are (i) the places where the animals are reared and (ii) the places where the records relating animal diseases are compiled. The two sources are actually called as prospective and retrospective sources, respectively. The prospective sources include herd/flock/farm household, slaughter houses and disposal centres /markets. The retrospective source includes veterinary clinic, diagnostic laboratories, artificial insemination centres, government departments and insurance organizations. As most of the livestock are confined to rural area, the data from the prospective source and that too from the household maintaining the livestock would be more reflective and worthwhile.

22. Methodologies for Impact Assessment of Livestock Diseases and Loss Projection Methods

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Public programs are designed to reach certain goals and beneficiaries. Methods to understand whether such programs actually work, as well as the level and nature of impacts on intended beneficiaries, are main themes of this book. Has the Grameen Bank, for example, succeeded in lowering consumption poverty among the rural poor in Bangladesh? Can conditional cash-transfer programs in Mexico and other Latin American countries improve health and schooling outcomes for poor women and children?
Does a new road actually raise welfare in a remote area in Tanzania, or is it a "highway to nowhere"? Do community-based programs like the Thailand Village Fund project create long-lasting improvements in employment and income or the poor?

Programs might appear potentially promising before implementation yet fail to generate expected impacts or benefits. The obvious need for impact evaluation is to help policy makers decide whether programs are generating intended effects; to promote accountability in the allocation of resources across public programs; and to fill gaps in understanding what works, what does not, and how measured changes in well-being are attributable to a particular project or policy intervention. Effective impact evaluation should therefore be able to assess precisely the mechanisms by which beneficiaries are responding to the intervention.

These mechanisms can include links through markets or improved social networks as well as tie-ins with other existing policies. The last link is particularly important because an impact evaluation that helps policy makers understand the effects of one intervention can guide concurrent and future impact evaluations of related interventions. The benefits of a well-designed impact evaluation are therefore long term and can have substantial spillover effects. This article also details challenges and goals in other realms of evaluation, including monitoring and evaluation (M&E), operational evaluation, and mixed-methods approaches combining quantitative and qualitative analyses. Broadly, the question of causality makes impact evaluation different from M&E and other evaluation approaches. In the absence of data on counterfactual outcomes (that is, outcomes for participants had they not been exposed to the program), impact evaluations can be rigorous in identifying program effects by applying different models to Survey data to construct comparison groups for participants. The main question of impact evaluation is one of attribution—isolating the effect of the program from other factors and potential selection bias.

Impact evaluation spans qualitative and quantitative methods, as well as ex ante and ex post methods. Qualitative analysis, as compared with the quantitative approach, seeks to gauge potential impacts that the program may generate the mechanisms of such impacts, and the extent of benefits to recipients from in-depth and group-based interviews. Whereas quantitative results can be generalizable, the qualitative results may not be. Nonetheless, qualitative methods generate information that may be critical for understanding the mechanisms through which the program helps beneficiaries.

Quantitative methods, focuses, span ex ante and ex post approaches. The ex-ante design determines the possible benefits or pitfalls of an intervention through simulation or economic

models. This approach attempts to predict the outcomes of intended policy changes, given assumptions on individual behavior and markets. Ex ante approaches often build structural models to determine how different policies and markets interlink with behavior at the beneficiary level to better understand the mechanisms by which programs have an impact. Ex ante analysis can help in reining programs before they are implemented, as well as in forecasting the potential effects of programs in different economic environments. Ex post impact evaluation, in contrast, is based on actual data gathered either after program intervention or before and after program implementation. Ex post evaluations measure actual impacts accrued by the beneficiaries because of the program. These evaluations, however, sometimes miss the mechanisms underlying the program's impact on the population, which structural models aim to capture. These mechanisms can be very important in understanding program effectiveness (particularly in future settings).

Although impact evaluation can be distinguished from other approaches to evaluation, such as M&E, impact evaluation can or should not necessarily be conducted independently of M&E. M&E assesses how an intervention evolves over time, evaluating data available from the project management office in terms of initial goals, indicators, and outcomes associated with the program. Although M&E does not spell out whether the impact indicators are a *result* of program intervention, impact evaluations often depend on knowing how the program is designed, how it is intended to help the target audience, and how it is being implemented. Such information is often available only through operational evaluation as part of M&E. M&E is necessary to understand the goals of a project, the ways an intervention can take place, and the potential metrics to measure effects on the target beneficiaries. Impact evaluation provides a framework sufficient to understand whether the beneficiaries are truly benefiting from the program—and not from other factors.

Different Evaluation Approaches to Ex Post Impact Evaluation

As discussed in the following chapters, a number of different methods can be used in impact evaluation theory to address the fundamental question of the missing counterfactual. Each of these methods carries its own assumptions about the nature of potential selection bias in program targeting and participation, and the assumptions are crucial to developing the appropriate model to determine program impacts. These methods, each of which will be understood and include

1. Randomized evaluations
2. Matching methods, specifically propensity score matching (PSM)
3. Double-difference (DD) methods
4. Instrumental variable (IV) methods
5. Regression discontinuity (RD) design and pipeline methods
6. Distributional impacts
7. Structural and other modelling approaches

These methods vary by their underlying assumptions regarding how to resolve selection bias in estimating the program treatment effect. Randomized evaluations involve a randomly allocated initiative across a sample of subjects (communities or individuals, for example); the progress of treatment and control subjects exhibiting similar preprogram characteristics is then tracked over time. Randomized experiments have the advantage of avoiding selection bias at the level of randomization. In the absence of an experiment, PSM methods compare treatment effects across participant and matched nonparticipant units, with the matching conducted on a range of observed characteristics. PSM methods therefore assume that selection bias is based only on observed characteristics; they cannot account for unobserved

factors affecting participation. DD methods assume that unobserved selection is present and that it is time invariant the treatment effect is determined by taking the difference in outcomes across treatment and control units before and after the program intervention. DD methods can be used in both experimental and nonexperimental settings. IV models can be used with cross-section or panel data and in the latter case allow for selection bias on unobserved characteristics to vary with time. In the IV approach, selection bias on unobserved characteristics is corrected by finding a variable (or instrument) that is correlated with participation but not correlated with unobserved characteristics affecting the outcome; this instrument is used to predict participation. RD and pipeline methods are extensions of IV and experimental methods; they exploit exogenous program rules (such as eligibility requirements) to compare participants and nonparticipants in a close neighbourhood around the eligibility cut off. Pipeline methods, in particular, construct a comparison group from subjects who are eligible for the program but have not yet received it. Finally, the handbook covers methods to examine the distributional impacts of programs, as well as modelling approaches that can highlight mechanisms (such as intermediate market forces) by which programs have an impact. These approaches cover a mix of different quantitative methods discussed in chapters 3 to 7, as well as ex ante and ex post methods. The handbook also draws examples and exercises from data on microfinance participation in Bangladesh over two periods (1991/92 and 1998/99) to demonstrate how ex post impact evaluations are conducted.

Disease projection: An epidemic of exotic disease in a livestock population can lead to substantial economic losses. An example is foot-and-mouth disease (FMD), a highly contagious disease of cloven-hoofed animals. The impact on a country's Gross Domestic Product from a 12-month FMD epidemic is estimated to be several Crores of Rupees over ten years. This includes the direct cost of controlling the disease (e.g., culling of infected animals and vaccination), and longer-term impacts such as the suspension of export markets due to the loss of FMD-free status. In the absence of within-country experience of a disease, computational modelling is an important tool for veterinary epidemiologists and disease managers to study the potential spread and impact of a disease, and evaluate control strategies. Modelling on a regional scale is complex due to large populations, heterogeneous herd types and farming practices, and regional differences in animal health policy. Some examples of the varied influences on the spread of disease in livestock include: • direct contact between animals (e.g., stock movements between farms, sale yards and abattoirs), • indirect contact between animals (e.g., via fomites transferred on vehicles, personnel and/or equipment), • viability of virus in the environment (influenced by relative humidity, temperature, UV radiation, etc.), • presence of vectors and conditions suitable for the establishment of vector-borne diseases, • pathogen characteristics (e.g., some viruses are species-specific while others span species), • climatic and seasonal factors (e.g., stock movement patterns may vary according to the time of year), • livestock management and market practices (can vary with species, farm type and region), • detection and reporting of disease (how long an epidemic progresses undetected is influenced by the willingness and timeliness of case reporting by owners, as well as the presence of surveillance programs), • disease control policies and the effectiveness of control measures (e.g., biosecurity practices, movement restrictions, vaccination, culling, treatment), • availability of resources to respond to an epidemic (e.g., animal health personnel, equipment, vaccine). Equation-based models are concise and well-established predictors for systems that are dominated by physical laws. Epidemics however, can be dynamically reshaped by irregular factors such as climate, economics, geography, psychology, sociology and jurisdiction-dependent animal health policies. The complex and variable environment in which a disease propagates can be

difficult to quantify mathematically. This is reflected in the adoption of individual-level models by veterinary epidemiologists, for example, microsimulations and agent-based systems. Individual level modelling is a paradigm shift away from top-down predictive mathematical algorithms. A population is viewed from the bottom up as comprising individuals with innate goals, logic and state. Interactions between individuals, and between individuals and the environment generate the model outcome in the form of emergent behaviour. The effect of variation, uncertainty and chance can be incorporated through stochastic techniques such as Monte-Carlo sampling. Hybrid models combine population-level modelling techniques with individual-level techniques. Models can be distinguished by how they handle time, space, variability, chance and uncertainty. However, these criteria do not lend themselves to a taxonomy. For example, a stochastic model may or may not be spatial, a deterministic model may view time discretely or continuously, etc. A simple classification scheme is thus proposed whereby models are grouped as population-level, individual-level or a hybridisation of both approaches (Figure 1). A hybrid approach is the cornerstone of the AADIS model of disease spread in livestock

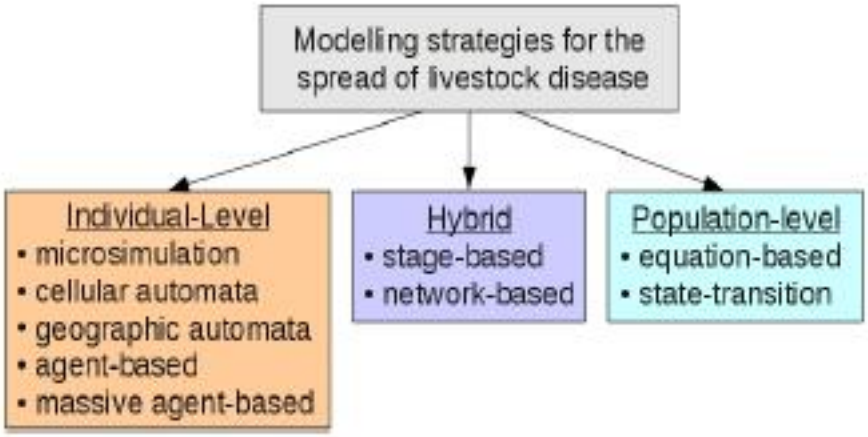

Figure 1. Modelling strategies.

23. Economic Impact of FMD in Indian Livestock

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Livestock is an important sub-sector of Indian agriculture contributing 25.6% to the agricultural gross domestic product (GDP) and has been consistently accounting for over 4.11% of the country's GDP (Department of Animal Husbandry, Dairying & Fisheries (DADF), Government of India, 2012-13). Livestock provides stability to income especially in the arid and semi-arid regions of the country and is an insurance against the vagaries of nature due to drought, famine and other natural calamities. The relevance of the livestock sector is further underlined by the National Agricultural Policy (NAP 2000) which emphasizes livestock as an important driver for achieving the targeted 4% growth in the agricultural sector by 2020.

The growth in livestock can be achieved by primarily focusing on nutrition and control of important livestock diseases, most importantly Foot and Mouth Disease (FMD). FMD is an acute highly contagious disease of cloven hoofed animals and causes high morbidity (up to 100%) and mortality particularly in young animals (50%) (Verma et al., 2012). India has FMD susceptible livestock population of 512 million (Department of Animal Husbandry, Dairying & Fisheries (DAHD&F), Government of India, 2012). The control of FMD is significant for protecting livestock and for improving livelihood and income generation for millions of farmers in the developing countries like India where FMD is endemic. In 2004, Government of India launched Foot and Mouth Disease Control Programme (FMD-CP) in 54 specified districts in eight states of the country out of 29 states and seven Union territories in the first phase with 100% central funding (for cost of vaccine, cold chain maintenance and logistic support) to undertake vaccination with the objectives to prevent economic losses due to FMD and development of herd immunity in cloven-footed animals (Singh et al., 2008). FMD causes huge loss not only to the animal owners but also to the nation as a whole due to ripple effect on the downstream and upstream stakeholders. The real challenge for the scientists is to understand the impact of the disease at the farm level and communicate the same to the policy makers, so as to extend appropriate support to animal health issues in priority setting. The magnitude of impact of a disease is an important guide for initiating appropriate action and hence it is imperative to estimate the direct losses.

Sampling frame

Sampling frame is very important for any impact analysis in order to estimate the losses and to generalize the results. Multistage random cluster sampling may be followed to collect primary data from sample farmers. At the first stage, few states of India may be selected purposively for the primary survey. In the second stage, three districts in each state were selected based on various geographical locations and FMD risk level. In the next stage, four clusters comprising five to seven village in each cluster may be identified randomly. In each of the selected clusters, the number of households owning bovine may be collected and the sample farmers to be surveyed in each clusters are to be proportionately allocated. In last stage, in each of the identified village, the number of livestock farms to be surveyed may be randomly selected.

Methodology to estimate losses due to FMD

Average loss due to milk yield reduction per animal

$$L_Y = 1/n \sum_{1}^{n} (E - A) * D * P$$

Where, L_Y =Average milk yield reduction per animal (Rs.)
E = Expected milk yield (Litres/day)
A = Actual milk yield during FMD period (Litres/day)
D = Duration of infection in in-milk animals
P = Price / litre of milk (Rs.)

Average loss due to draught power reduction per animal

$$L_D = 1/n \sum_{1}^{n} (D * adj) * W$$

Where, L_D = Loss due to draught power reduction per animal(Rs.)
D = Disease persistency in the bullocks (number of days)
W = Hiring charges / day (Rs.)
n = Number of bullocks recovered from FMD
adj = Adjustment factor

Average treatment costs per animal

$$L_T = 1/n \sum_{1}^{N} (F + M + I)$$

Where, L_T =Average treatment costs per animal (Rs.)
F = Fees for veterinarians / farm (Rs.)
M = Cost of medicines / farm (Rs.)
I = Cost of indigenous treatment during the infected period (Rs.)
N =Number of farms
n = Total number of animals infected by FMD

Average extra labour engaged for nursing the affected animal

$$L_{EL} = 1/n \sum_{1}^{N} [(M_{Post} - M_{Pre}) / 8] * D * W$$

Where, L_{EL} =Average extra labour engaged for nursing the animal (Rs.)
M_{post} = Manpower during FMD period (hours/day)
M_{pre} = Manpower during pre-FMD period (hours/day)
D = Duration of infection (days)
W = Wage Rate / day (Rs.)
N =Number of farms
n = Total number of animals infected by FMD

Average loss due to distress sale per animal

$$L_s = 1/n \sum_{1}^{n} (A - S)$$

Where, L_S =Average loss due to distress sale per animal (Rs.)
A = Market value of animals before FMD (Rs.)

S =Sale value of animals after FMD (Rs.)
n = Total number of animals infected by FMD and sold

Average loss due to mortality per animal

$$L_M = 1/n \sum_{1}^{n} A_j * V_j$$

Where, L_M =Average loss due to mortality per animal in indigenous cattle (Rs.)
j = Category of animals, viz. In-milk, dry, bull, bullocks, immature males, heifer, male calf and female calf
A_j =Number of animals in different categories
V_j = Average value of animals (Rs.)
n = Total number of animals infected by FMD and died

Total loss per animal

$$E_T = L_Y + L_D + L_T + L_{EL} + L_S + L_M$$

Where, E_T = Total loss per animal (Rs.)
L_Y = Average loss due to milk yield reduction per animal (Rs.)
L_D = Average loss due to draught power reduction per animal (Rs.)
L_T = Average treatment costs per animal (Rs.)
L_{EL} =Average extra labour engaged for nursing the animal (Rs.)
L_S =Average loss due to distress sale per animal (Rs.)
L_M =Average loss due to mortality per animal (Rs.)

Conclusion

- Economic impact assessment helps to quantify the lossess due to a disease.
- The losses may be estimated at farm level, state, region or national level.
- The estimates guides the policy makers for designing appropriate control options.
- It also helps to rationalize the research investment in the livestock health.

24. Loss Assessment Based on Secondary Data-Peste des petits ruminants (PPR) in Sheep in India

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Peste des petits ruminants (PPR) is an acute and highly contagious viral disease in sheep and goats. It is an OIE notified transboundary disease causing severe morbidity and mortality [Diallo et al, 2007; Venkataramanan et al, 2005]. The clinical symptoms include pyrexia, stomatitis, discharge through ocular and nasal orifices, enteritis and pneumonia. PPR was first reported in the Ivory Coast of West Africa and later found in other parts of the world including India. In India, PPR was first recorded in the Tamil Nadu state during 1987 and was later in other states. PPR is enzootic in India and outbreaks occur regularly among small ruminants population [Kerur et al, 2008; Singh et al 2004]. Despite the significance of the disease among small ruminant population in India, the reliable loss estimates are still lacking.

Materials and Methods

The loss due to PPR in sheep and goat was estimated based on the data derived from secondary sources, expert opinion, field investigation results and past reviews. The details of the data sources for PPR loss estimation are presented in Table 1.

Table 1. Parameters considered for assessing the loss due to PPR in sheep and goats

Label/ Identity	Parameters	Sheep	Data source
A	Population	Actuals	2012 livestock census
A1	Adults (%)	Actuals	2012 livestock census
A2	Young (%)	Actuals	2012 livestock census
B	Incremental PPR prevalence *(percent/year)	5 or 10	Awase et al 2013; Singh et al 2004 and Balamurugan et al 2011
C	Adult Mortality (%)	10	Expert opinion
D	Adult Morbidity (%)	5	Expert opinion
E	Lamb/kid Mortality (%)	20	Expert opinion
F	Lamb/kit Morbidity (%)	20	Expert opinion
G	Average weight of Adult (kg)	20	Probable values
H	Average weight of Young (kg)	10	Probable values
I	Price of live weight animal (Rupees/kg)	300	Prevailing market price in Karnataka during 2013
J	Price of the one young animal (Rupees)	3000	
K	Price of the one adult animal (Rupees /Kg)	Rs. 6000/-	

L	Reduction in body weight due to morbidity (%)	10	Obtained during outbreak investigation in Karnataka state
M	Average number of Lamb/lambing or kid /kidding per animal	1	Expert opinion
N	Average birth weight of lamb/kit (Kg)	2.5	Expert opinion
O	Proportion of female animals in abortion (%)	2	Expert opinion
P	Inter-kidding or Lambing period (months)	10	Expert opinion
Q	Delay in next conception (months)	3	Expert opinion
R	Cost of feed incurred (Rupees per animal/month)	120	Obtained during outbreak investigation in Karnataka state
S	Additional period of feeding the animals (months)	3	Expert opinion
T	Average treatment cost of the animals (Rupees)	125	10 % increment of Awase et al 2013 observation
U	Increased cost of Management including labour (Rupees)	60	Obtained during outbreak investigation in Karnataka state
V	Other miscellaneous indirect cost (Rupees)	60	Obtained during outbreak investigation in Karnataka state

*considered based on Awase et al 2013 incidence percentages and the incremental prevalances from Singh et al 2004 and Balamurugan et al. 2011

Models to assess the direct and indirect losses due to PPR in Sheep

1) Losses due to mortality in sheep

I. M_{AS}= A x A1x B x C x G x I

Where M_{AS}=Loss due to mortality in adult sheep (Rs.)
A=Population of sheep
A1= Adult population (%)
B= Incremental PPR prevalence (%)
C= Adult mortality (%)
G= Average weight of adult (Kg)
I= Price of live weight animal (Rs./Kg)

II. M_{YS} = A x A2 x B x E x H x I

Where M_{YS}=Loss due to mortality in young sheep (Rs.)
A=Population of sheep
A2= Young animal population (%)
B= Incremental PPR prevalence (%)
E= Young animal mortality (%)
H= Average weight of young one (Kg)
I= Price of live weight animal (R.s/Kg)

2) Losses due to body weight in sheep

a) Direct loss due to reduction in body weight in sheep

I. BW_{AS} = A x A1x B x D x G x L x I

BW_{AS}= Loss due to body weight in adult sheep (Rs.)

A=Population of sheep

A1= Adult population (%)

B= Incremental PPR prevalence (%)

D= Adult morbidity (%)

G= Average weight of adult (Kg)

L= Reduction in body weight due to morbidity (%)

I= Price of live weight animal (Rs/Kg)

II. BW_{YS} = A x A1x B x F x H x L x I

BW_{YS}= Loss due to Body weight in Young sheep (Rs.)

A=Population of sheep

A2= Young animal population (%)

B= Incremental PPR prevalence (%)

F= Young animal morbidity (%)

H= Average weight of young animal (Kg)

L=Reduction in body weigh due to morbidity (%)

I= Price of live weight animal (Rs/ Kg)

b) Live weight loss due to Increased inter-lambing period

Reduction in number of lambs due to lengthy inter-lambing period after infection with a disease caused loss in the live body weight. Such losses were estimated by

BW_{ILP} = A x A1x B x0.5 x { [12/P]- [12/P+Q] x M x N x I , which is slight modification of earlier methods (Singh and Prasad 2009)

BW_{ILP} = Cost of Body weight loss due to increased inter lambing period in adult female sheep (Rs.)

A=Population of sheep

A1= Adult population (%)

B= Incremental PPR prevalence (%)

P= Inter-kidding period (months)

Q= Delay in next conception (months)

M= Average number of lamb/lambing

N= Average birth weight of lamb

I= Price of live weight animal (Rs./Kg)

c) Live weight loss due to Increased Abortion

BW_{IA} = A x A1x B x0.5 x O x N x I

BW_{IA}= Cost of Body weight loss due to increased abortions in female sheep (INR in Million)

A=Population of sheep

A1= Adult population (%)

B= Incremental PPR prevalence (%)

O= Proportion of female animals in abortion in percentage or increased abortion rate in percentage

N= Average birth weight of lamb (kg)

I= Price of live weight animal (Rs./Kg)

3) Other Associated loss

The associated loss due to PPR infection were assessed based on assumptions derived from literatures &/or discussion with the experts

a) Cost of high feeding and rearing inputs

I. in Adults sheep

FRI_{AS}= A x A1x B x D x R x S

FRI_{AS}= Cost of high feeding and rearing inputs in adult sheep (Rs.)
A=Population of sheep
A1= Adult population (%)
B= Incremental PPR prevalence (%)
D= Adult Morbidity (%)
R=Price of feeding per animal/month in that particular period
S= Additional Period of feeding the animals in month

II. in Young sheep

FRI_{YS}= A x A2x B x F x R x S

FRI_{YS}= Cost of high feeding and rearing inputs in Young sheep (Rs.)
A=Population of sheep
A1= Adult population (%)
B= Incremental PPR prevalence (%)
F= Young animal morbidity (%)
R=Cost of feed incurred per animal/month
S= Additional Period of feeding (months)

b) Miscellaneous loss

I. in Adults sheep

Mis_{AS}= A x A1x B x D x (T+U+V)

Mis_{AS}= Cost of miscellaneous loss in Adult sheep (Rs.)
A=Population of sheep
A1= Adult population (%)
B= Incremental PPR prevalence (%)
D= Adult morbidity (%)
T= Treatment cost of the animals (Rs.)
U= Increased cost of management including labour (Rs.)
V= other miscellaneous indirect cost (Rs.)

II. in Young sheep

Mis_{YS}= A x A2x B x F x (T+U+V)

$Misy_S$= Cost of miscellaneous loss in young sheep (Rs.)
A=Population of sheep
A1= Young animal population (%)
B= Incremental PPR prevalence (%)
D= Young animal morbidity (%)
T= Treatment cost of the animals (Rs.)
U= Increased cost of management including labour (Rs.)
V= other miscellaneous indirect cost (Rs.)

Suggested Reading

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